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Version: Version of Record

Link(s) to article on publisher's website:

<http://dx.doi.org/doi:10.21954/ou.ro.0000d4d3>

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**CHANGES IN JOINT LAXITY  
ASSOCIATED WITH THE MENSTRUAL  
CYCLE, WITH PREGNANCY, WITH THE  
POST-PARTUM PERIOD, AND WITH  
THE MENOPAUSE.**

**Stephen Eric Sandler DO FE Cert**

**A thesis submitted in fulfilment of the requirements for the  
degree of Doctor of Philosophy at the Open University**

**2006**

**THE BRITISH SCHOOL OF OSTEOPATHY  
LONDON**

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DATE OF SUBMISSION 26 JANUARY 2005

DATE OF AWARD 16 MARCH 2006

## **ABSTRACT**

This thesis looks at changes in joint laxity associated with the menstrual cycle, pregnancy, and the menopause and the potential contribution of female hormones to that laxity.

A pilot study revealed that there was a potential link between the phases of the menstrual cycle and the onset of musculo-skeletal injury.

Following an extensive literature search studying the various hormones involved in the menstrual cycle, and their receptors in musculo-skeletal tissues, an interesting picture emerged that appeared to support the evidence evinced in the pilot study. This was particularly so with regard to papers that attempted to show a link between athletic injury and the phases of the menstrual cycle.

Four experiments were designed using a Hyperextensometer capable of examining the range of movement in the first metacarpo-phalangeal joint of the subject's dominant hand. Women with a normal menstrual cycle, pregnant women, breast feeding women, and women after the menopause, were all examined to discover if their hormones had an effect on the mobility of the joint concerned.

The results of the menstrual cycle study showed that every subject underwent an increase in laxity as she progressed throughout the cycle, and that change was particularly significant in the first phase of the cycle. The pregnancy study showed that once again all subjects measured showed an increase in joint laxity as the pregnancy progressed, however this laxity change was neither uniform throughout the pregnancy nor the same from one subject to another.

The majority of subjects however experienced an increase in laxity in the middle months of their pregnancies. The study found that when comparing primiparous to multiparous subjects the primiparous group changed earlier. The post natal study looked at changes in laxity that took place in breast versus bottle feeding mothers. It failed to draw any significant conclusions from the data. Finally the menopause study showed that women who take a hormone replacement retain joint laxity more than a control group. The implications of these findings were discussed.



## **ACKNOWLEDGEMENTS**

I am eternally grateful to my supervisors Dr Martin Collins and Professor Roger Woledge not only for their academic guidance and tutorial support but for their belief in me and in this study. Without their belief this work would not have been completed.

I would like to thank Dr Mark Johnson for his help and suggestions in the second part of the study and for providing the laboratory facilities for analysing the blood samples on my behalf.

I would like to thank Dr Adam Collins for his teaching and patience in guiding me through the statistical analyses.

I am indebted to all the subjects who were extremely generous in allowing me to invade their lives at evenings and weekends to take samples and perform measurements. Again without their generosity and support this work would not have been possible.

Last but not least I would like to thank my wife and family for giving me the space, time, and support to finish what for me has been a challenge at every level.

This thesis is dedicated to them

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# ***Introduction***

"Structure governs function" is a key tenet in osteopathic philosophy.

According to Stone this is better written as: "motion relates to physiology"

(Stone 1999) .

## **G.R.E.N.R.I.M.**

Growth

Reproduction

Excretion

Nutrition

Respiration

Irritability

Movement

These are the characteristics of biological life. Every living thing, and every cell within every living thing possesses and displays these characteristics, and no one of them is any more important to life than any other. However, the presence or absence of motion is often used as a defining characteristic within medicine, as for example when a paediatrician uses the APGAR score when a child is born. He assesses the heart rate via the pulse, the breathing rate and effort via motion in the chest, and the degree of activity or motion in the child in general., together with the child's muscle tone. All of these factors use motion as an indicator of normality in that child.

We understand death to be the ultimate absence of motion at a macro and a micro level.

The process of autoregulation ensures that the organism minimises cell damage by allowing motion only within pre-set physiological parameters. Using the example of a synovial joint, quality and quantity of motion are governed by the shape, plane, and integrity of the articular surfaces. The joint capsule and the intra- and extra-articular ligaments are designed to limit motion at the end of the joint's normal physiological range. The skeletal muscles and their accompanying tendons guide motion and control the speed of motion as well as providing postural stability and control. The force, speed and direction of pull of the muscles all contribute to the control of motion. The central nervous system controls the whole, relating the postural and proprioceptive functions to the body's desire for motion. All of these factors are controlled by a remarkable system of hormones, enzymes, and other biological and psychological factors which contribute towards motion control. The qualitative assessment of motion can be further sub-divided into three categories: hypomobility, normal mobility, and hypermobility.

This thesis looks at one of the factors that may be responsible for changing and governing motion control in a specific selected joint in female subjects.

During more than 25 years of osteopathic clinical practice the author has noticed that some women, particularly during their reproductive years, have a tendency to musculo-skeletal instability. Often a relatively minor trauma will produce an unexpected and painful reaction in the musculo-skeletal system that is apparently out of all proportion to the magnitude of the trauma. For example, she may bend down to pick up a book, and her low lumbar muscles will go into spasm. At other times she is able to lift, bend, and carry shopping

with little effect. The trauma rarely produces long-lasting or serious damage; usually it is simply a spinal apophyseal joint overstrain, or a simple painful but short-lived muscle spasm. Often, if it is a chronic condition, she hurts the site of an old injury, rarely initiating a new problem.

The fact that many of these patients were female and these incidences occurred during their reproductive years led the author to seek a potential link between these facts. Was there a relationship between the incidence of pain and the phases of her menstrual cycle? On an anecdotal level, some women report that they suffer musculo-skeletal pain when they are at mid-cycle or when they are pre-menstrual., but there is little evidence to date to suggest that this might be linked to a change in mobility in the articular system.

The thesis begins with a pilot study investigating the incidence of acute chronic and recurrent musculo-skeletal pain during the different phases of the menstrual cycle as seen in osteopathic practices in the United Kingdom.

This led on to a series of studies investigating laxity at different stages in the female life cycle. The first study involved women who had an accurate and predictable menstrual cycle, the second study investigated women during pregnancy, the third study investigated laxity in women who were lactating, and the fourth study investigated women after the menopause.

The results of these studies and the conclusions drawn are then critically reviewed.

## **Aims of the thesis**

The main aim of the thesis is to investigate joint mobility in women. At different times within a woman's life for, example during the menstrual cycle, or during pregnancy, the profile of the sex hormones such as oestrogen and progesterone in her blood will change according to physiological limits. The hypothesis is that these sex hormones have a potential effect on the tissues regulating mobility in the articular system and, that the mobility of the joints has the potential to change during the various phases of a woman's reproductive life.

## **Subsidiary aims of the thesis**

The first subsidiary aim of the thesis is to investigate musculo-skeletal pain incidence during the menstrual cycle, the timing of the onset of pain in relation to the days of the cycle, and the chronicity of that pain as seen in patients presenting for treatment in osteopathic practices throughout the United Kingdom. The hypothesis is that women report musculo- skeletal pain at specific points in the menstrual cycle with greater frequency than at other times and this is related in some way to the phases of the cycle.

The second subsidiary aim of the thesis is to carry out a series of experiments on women during different phases of reproductive life to investigate mobility at the first metacarpo-phalangeal joint of the subject's dominant hand. The

hypothesis is that joint mobility can change under the influence of the sex hormones, and that as the levels of these hormones change during the different phases of her reproductive life, therefore potentially so does her mobility.

If the women do report pain with greater frequency at different phases of the menstrual cycle, is this then related to changes in joint mobility itself governed and affected by changes in the levels of the circulating sex hormones?

# ***Chapter One:***

## ***The Pilot Study***

## **Introduction**

A pilot study was undertaken to explore the potential links between the menstrual cycle and musculo-skeletal pain patterns reported in osteopathic practices in the UK. Three preliminary surveys were distributed, reviewed, and modified over a six month period. Patients were drawn from the author's private practice. The purpose of this preliminary work was to refine the questions used in the main survey itself.

It was found from the preliminary surveys that one factor that seemed to be consistent amongst these women was the phase of the menstrual cycle at the time of the injury. Because of these findings, a much larger sample group from all around the UK was studied to investigate the potential association between the phases of the menstrual cycle and the incidence of musculo-skeletal pain or injury.

## **Literature Review**

A review of the literature using the current Medline database, the National Library of Medicine database and OVID online, showed that pain thresholds change in relation to the phases of the menstrual cycle. Neurotransmitters and neuropeptides of the central nervous system can also be affected by the changes in levels of the sex hormones. The review then studied incidence of athletic injury and in female athletes,



because this would also be relevant when looking at pain incidences and the menstrual cycle as found in osteopathic practice.

Pain thresholds have been found to change at different times within the menstrual cycle. In menstruating women, pain thresholds are highest in the mid-menstrual phase, and in those women who take oral contraceptives, pain thresholds were highest at the pre- and mid-menstrual phases (Rao et al., 1987).

The menstrual cycle can also modify the number of tender points noted on palpation in patients who suffer from fibromyalgia. The number of tender points was greater in the follicular phase of the cycle, as compared to the luteal phase in women who were not using oral contraceptives (Hapidou et al., 1998).

Patients who suffer from pain in the muscles of mastication have also been studied to see if the ovarian hormones have a part to play in their pain thresholds. The conclusion here was that the pain thresholds are influenced by the ovarian cycle, but only to a minor extent (Cimino et al., 2000).

Temporomandibular joint pain rises towards the end of the cycle and peaks during menstruation when compared with the control groups and with males (Le Resche et al., 2003).

It has been postulated that the effect on pain thresholds may be due to an effect of the sex hormones on the central nervous system (CNS).

Oestradiol, progesterone and some of their metabolites modulate the activity of neurotransmitters and neuropeptides in the CNS (Kuhl, 2002).

The distribution and concentration of sex steroids in the various CNS regions is partly dependant on the serum levels, but also on the local synthesis of the steroids. In general oestradiol and testosterone exert a stimulatory effect, and progesterone an inhibitory effect, on neuronal activities which are mediated by excitatory and inhibitory amino acids and neuropeptides. Somatic complaints such as back pain and abdominal pain are highest before and during menstruation and have been found to be associated with a lowered pain threshold due to a fall in beta endorphins within the central nervous system (Straneva et al., 2002).

It is not just pain thresholds in general that can change but also the thresholds of different types of pain. Pressure pain thresholds, pinch pain thresholds, heat pain thresholds and tactile thresholds will vary between the sexes, and they also vary in different phases of the cycle and from woman to woman (Bajai et al., 2001).

Thus it has been demonstrated that women will report different levels of pain at various phases of the menstrual cycle. This is probably due to the effect of the changing levels of sex hormones on the part of the central nervous system responsible for reporting these different sensitivities (Straneva et al., 2002).

There have been studies investigating the use of oral contraceptives and musculoskeletal pain. Oral contraceptives cause attenuated muscle soreness in subjects performing an exhaustive stepping activity in the laboratory when compared to a control group (Thompson et al., 1997). Women using the oral contraceptive had a lower rate of injuries

compared to women who were not using them. The authors believed that the results were explained by the observation that oral contraceptives ameliorate some symptoms of the premenstrual and menstrual phases of the cycle which might also affect coordination and hence the risk of injury (Moller-Nielsen and Hammar, 1989).

In the study on patients with temporomandibular pain referred to above, women using oral contraceptives were compared to those not using “the pill” and they both showed an increase in pain in the menstrual phase compared to control groups; however, the “non pill” users also showed a second peak of pain around days 13-15 (Le Resche et al., 2003). It is significant that this is the ovulatory phase when the levels of oestrogen rise to a peak before falling thus allowing the pre-ovulatory surge of lutenising hormone.

## **Athletic injury and the menstrual cycle**

With the participation of women in athletics growing rapidly over the last two decades, a disturbing gender specific pre-disposition has emerged regarding anterior cruciate ligament (ACL) injuries. (Arendt, 1995; Slauterbeck, 1995; Teitz, 1997; Wojtys, 1998; Hewett, 2000; Slauterbeck, 2001; Toth, 2001; Arendt, 2002; Slauterbeck, 2002). Female athletes have a two to eight-fold higher incidence of ACL injury than their male counterparts (Toth and Cordasco, 2001). It has been estimated that

80,000 women will sustain ACL tears per year (Arendt, 2001; Arendt et al., 2002.). The majority of these injuries occur by non-contact mechanisms, most often during landing from a jump or making a lateral pivot whilst running.

The risk factors for these types of injury may be categorised as intrinsic (anatomical. histological. biochemical. and hormonal) or extrinsic (environmental or biomechanical).

The level of female sex hormones oestrogen, progesterone and relaxin fluctuate physiologically during the menstrual cycle and are reported to increase ligamentous laxity; thus they are a possible cause of decreases in both passive and active knee instability in female athletes (Slauterbeck and Hardy, 2001; Deie et al., 2002; Slauterbeck et al., 2002). It is therefore appropriate to consider whether there is a relationship between the phases of the cycle when assessing mechanisms of ACL injuries in female athletes (Wojtys et al., 2002).

Target cells for oestrogen and progesterone were first identified in the fibroblasts of the human ACL as early as 1996 (Liu et al., 1996). This study, using immunohistochemical techniques, localised both oestrogen and progesterone receptors in a series of tissues from both male and female human subjects. All ligament specimens were obtained at surgery. Both oestrogen and progesterone receptors were localised to synoviocytes in the synovial lining of the knee joints fibroblasts in the ACL stroma, and cells in the blood vessel walls supplying the knee joints, suggesting that female sex hormones have an effect on the structure and composition of these tissues.

In 1998, a more sophisticated method of reverse transcription-polymerase chain reaction was used to evaluate the expression of oestrogen and progesterone receptors in ligament tissues from male and female rabbits and male and female humans. The presence of these receptors in the knee ligaments were found in significant numbers, with female subjects having a proportionally larger volume of receptors when compared to males (Sciore et al., 1998).

Relaxin receptors were finally identified in the human ACL in 2003 (Dragoo et al., 2003).

Remnants of ACL were harvested from men and women undergoing routine ligament reconstruction. Relaxin was biotinylated [Biotinylation: A technique whereby biotinyl groups are incorporated into a molecule in vitro to visualise specific substrates by incubating them with biotin labelled probes] and bioassayed for biological activity with the use of the mouse inter-pubic ligament. Samples from the women but not from the men showed uniform specific binding that was limited to synovial lining cells, stromal fibroblasts and cells lining blood vessels. These results indicate that relaxin exhibits specific binding in the female anterior cruciate ligament and again seem to support the hypothesis that sex hormones have a part to play in the rupture of these ligaments in female athletes because the presence of relaxin receptors allows the hormone to weaken the ligament (Dragoo et al., 2003).

Thus a greater proportion of receptors for oestrogen, progesterone and relaxin all exist within the ACL in females than males (Sciore et al., 1998).

A number of studies have looked into the effect of sex hormones within the phases of the menstrual cycle, specifically on fibroblast proliferation and pro-collagen levels, as an indicator of collagen synthesis (Liu et al., 1997; Yu et al., 1999; Rasanen and Messner, 2000; Yu et al., 2001).

Collagen synthesis was found to be significantly reduced with increasing oestradiol concentration. On days 1 and 3 of the menstrual cycle, there was a dose-dependent decrease in the proliferation of ACL fibroblasts with increasing oestradiol concentrations. This dose-dependent effect of decreased fibroblast proliferation with increasing oestradiol concentration became less apparent at 7, 10, and 14 days. On days 1 and 3 pro-collagen synthesis decreased in a dose-dependent manner with increasing oestradiol concentrations. This decrease contributes to the great increase in joint laxity observed during the first phase of the cycle. On days 7, 10, and 14, this dose-dependent effect was attenuated as the concentrations of oestradiol increased. An increase in fibroblast proliferation and pro-collagen type 1 synthesis was observed with increasing progesterone concentration. However, the effect was more pronounced at lower oestrogen concentrations, suggesting that oestrogen levels were the dominant factor (Liu et al., 1997; Yu et al., 1999).

Pregnancy is one time when ligaments change under the influence of sex hormones such as relaxin and oestrogen. Two studies have looked at the ACL specifically during pregnancy (Blecher and Richmond, 1998; Charlton

et al., 2001), and the results support the hypothesis. The first study noted that substantial remodelling from large diameter to small diameter collagen fibres occurred in the ACL graft ligament in a woman who had undergone ACL reconstruction two months before conception. The authors considered this was due to the relaxin levels, but did not appear to correlate this with any blood analysis for the hormone. The second study was more specific and looked at both ACL translation as measured using a specific arthrometer test, and then correlated these measurements with oestradiol levels during the third trimester of the pregnancy and the post-partum period. The results showed that high serum oestradiol levels during the third trimester of pregnancy correlate with increased anterior tibial translation and that this anterior tibial translation decreases with the return of the serum oestradiol to non pregnant levels.

Not all authors agree with these conclusions.

In a study in 2002 by Arnold et al., using an arthrometer, knee joint laxity was measured in 57 female athletes and 5 men in a 4-week period.

Analysis of variance of the mean readings between the groups revealed a significant change in weekly serum relaxin levels, but not in anterior knee translation. Regression analysis also failed to find a relationship between the variables. Therefore they concluded that relaxin does not have an effect on knee laxity (Arnold et al., 2002). Although the presence of sex hormones, particularly oestrogen, may predispose female athletes to higher injury rates, they did not find the higher injury rates correlated with changes in ACL laxity.

Other researchers, ( Wojtys et al., 1998; Arendt et al., 2002; Wojtys et al., 2002), have different conclusions regarding the relevance of non-contact ACL injuries and the timing of the cycle .

To summarise, there appears to be abundant but contradictory evidence to suggest that the cyclic sex hormones may have a part to play in determining at which point in the menstrual cycle women might present with pain to osteopathic practices.

In order to examine these hypotheses further, a study was undertaken researching into the pain presentation patterns of women presenting to osteopathic practices in the UK.

## **Method**

A simple questionnaire was designed, piloted, and amended three times between 1993 and 1994, before the final version was felt ready to be distributed. The subjects for this preliminary work all came from the author's private practice. Five hundred practitioners were chosen at random by polling one practitioner in three from the register of The General Council and Register of Osteopaths. Each practitioner was sent two copies of the questionnaire, and asked to complete them for the next two female patients of menstruating age presenting to the practice with musculo-skeletal pain. Each patient taking part in the study was given an information sheet (see appendix) and given the opportunity to withdraw



from the study if they did not wish to take part. Ethical approval for the study was obtained from the Ethics Committee of The British School of Osteopathy.

## **The Questionnaire**

### ***1. The age of the patient***

### ***2. The length of the menstrual cycle***

### ***3. Present problem (please tick the predominant complaint)***

***Low-back pain***

***Sacro-Iliac or Pelvic joint pain***

***Cervical spine pain***

***Peripheral joint pain***

***Other pain (please specify)***

### ***4. Chronicity of the pain (please tick one of the following)***

***Acute onset***

***Chronic pain***

***Recurrent acute onset***

### ***5. When did the pain start in relation to the last menstrual cycle (i.e. when was the last period in relation to the pain.)***

## **Comments on the questionnaire:**

Instructions to practitioners included the following points of additional information designed assist them in their participation in the study.

#### Question 2: The length of the menstrual cycle

If the subject admitted to not having a regular cycle, as defined by her, she was excluded from the study and the osteopath was asked to find another subject. Subjects were also excluded if they were using any hormone preparations such as Hormone Replacement Therapy (HRT), an oral contraceptive, or hormone implants as it was thought that the menstrual cycles of these subjects were not under the control of a natural hormone profile.

The length of the cycle and the predictability of a regular cycle were deemed to be very important to the outcome of the study. In a regular menstrual cycle of 28 days, ovulation occurs in the middle of the cycle. The luteal phase of the cycle is fixed and constant so that 14 days after ovulation menstruation will occur (Johnson et al., 1994). Therefore the relationship of pain to the cycle can only be accurately ascertained if only those women with a predictable and regular cycle are included in the study.

#### For Question 4: Chronicity of pain

This question is particularly important when looking at incidences of acute chronic or recurrent acute pain. Is the character of the pain related to one particular phase of the cycle?

#### For Question 5: When did the pain start in relation to the menstrual cycle?

This was not thought to be an easy question to answer in every case so the osteopath was advised to apply the following formula.

“Determine where she is now in her cycle, and subtract the number of days she has had pain in order to calculate the day that pain started in her cycle”.

I.e. if she has had 9 days of pain, and she is on day 6 of her menstrual cycle counting the first day of bleeding as day one, then the pain occurred on day 25 of a 28-day cycle.

## **The Hypotheses**

The experimental hypothesis states that there is a relation between the incidence of pain and specific days of the menstrual cycle. The null hypothesis states that pain is equally likely to occur randomly within the menstrual cycle.

The second experimental hypothesis states that there is an increased risk at specific points within the cycle of specific sites of pain such as low-back pain, cervical spine pain, upper extremity, and lower extremity pain.

The second null hypothesis is that these specific sites of pain are independent of specific days within the cycle and are just as likely to occur at one time within the cycle as at any other.

The third experimental hypothesis states that the chronicity of pain occurring within the menstrual cycle is again related to specific dates within the menstrual cycle. The null hypothesis is that acute chronic and

recurrent pain is just as likely to occur at one point within the cycle as at any other.

**Results and statistical analysis**

Altogether 304 responses were received out of the 1,000 questionnaires sent out and the mean age of the responders was 34 ( $\pm$  SD 7.43) years. One was rejected for further analysis as it referred to day 36 of a subject's menstrual cycle. The other 303 incidents were divided into five categories:

Table 1: Area of Pain and Incidences

Area of Pain	Incidences
Low-back	109
Cervical Spine	67
Upper Extremity	25
Lower Extremity	28
Other Pains	74

The category "other pain" included responses indicating sacro-iliac joint pain and buttock pain. It was felt that there was not sufficient scope in the questionnaire to justify including these as either a separate category or combined with the low-back pain category.

Analysis of “All Pain”

Figure 1 shows the frequency of incidence of all pains being reported in each three-day time band starting from day one of the subject’s menstrual cycle. This was the first day of menstrual bleeding.

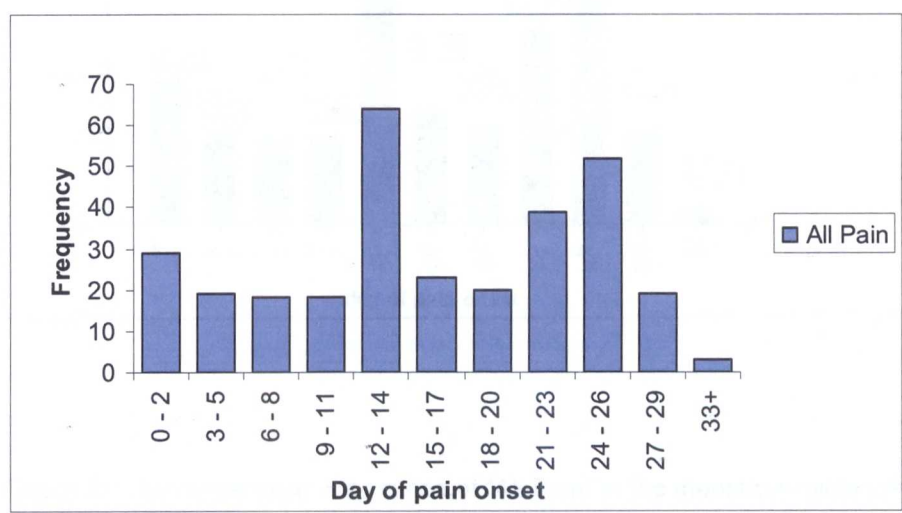


Figure 1: The Frequency of “All Pain” in the menstrual cycle (n=303)  
These results illustrate that a higher frequency of pain was reported around days 12-14 and days 21-23 and 24-26.

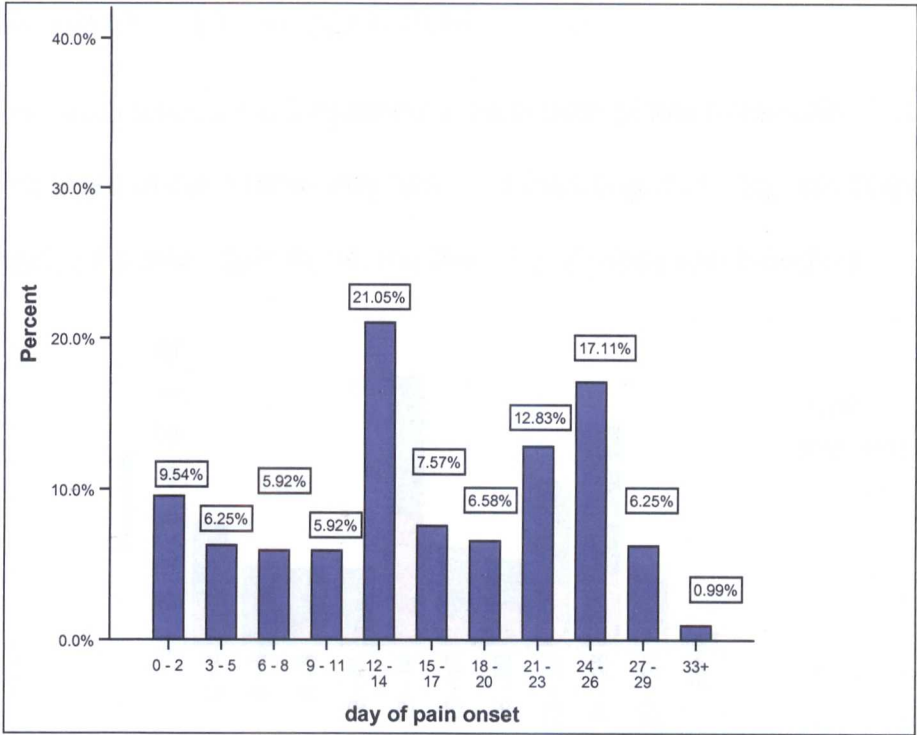


Figure 2: The percentage occurrence of “All Pain” in the menstrual cycle (n=303)

As shown in figure 2 these frequencies represented 21.05% 12.83% and 17.11% of the totals recorded respectively. At other times in the cycle this did not exceed 8% except for days 0-3 where the peak was around 9.54%

Using a  $\chi^2$  test on the data ( $\chi^2 = 111.018$ ,  $df = 10$ ) the results were found to be significant ( $p < 0.001$ ). This shows that there is a relationship between the day of the menstrual cycle and the onset of pain. Hence the null hypothesis that pain is likely to occur with the same frequency anywhere in the cycle can be rejected.

The results were then analysed to determine if specific sites of pain were associated with the days of pain onset during the menstrual cycle.

Analysis of Low-back Pain

Figure 3 shows the frequency of incidence of low-back pain alone being reported in each three-day time band starting from day one of the subject's menstrual cycle, the first day of menstrual bleeding.

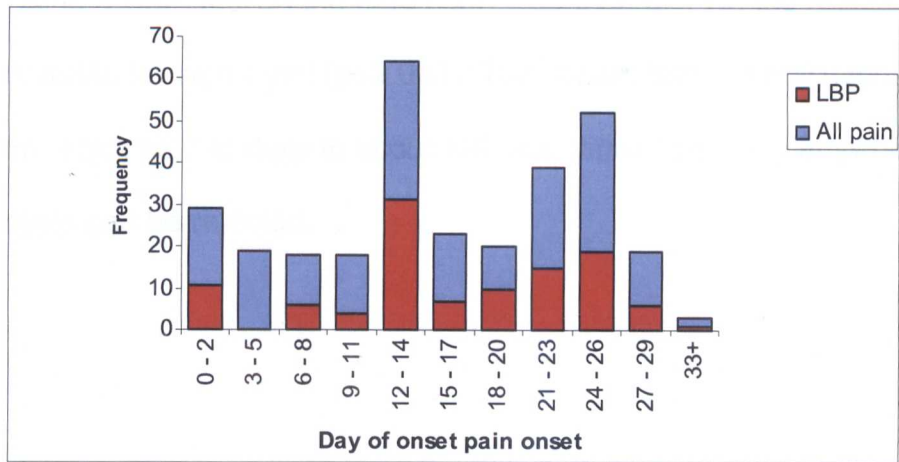


Figure 3: Incidence of “Low-back Pain “as displayed with “All pain” in the menstrual cycle (n=109).

These results illustrate that a higher frequency of incidences were reported around days 12-14, days 21 to 23 and days 24-26.

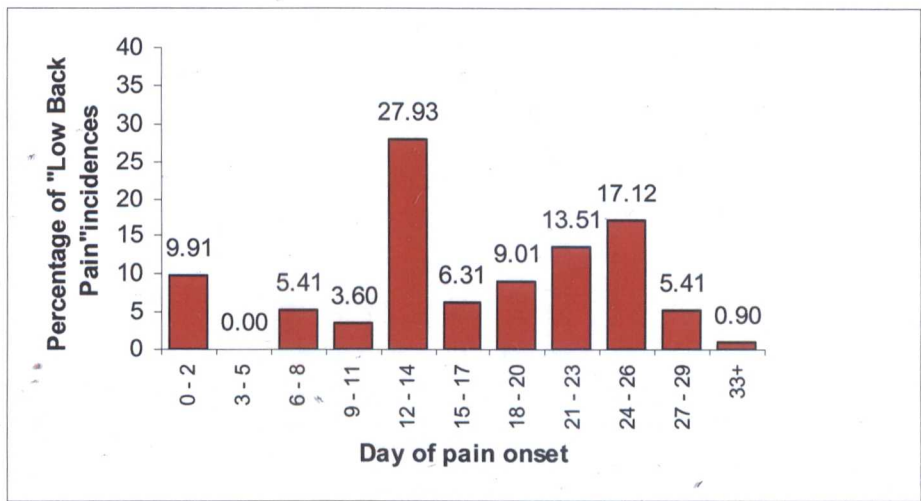


Figure 4: The percentages of “Low-back Pain “incidence in the menstrual cycle (n=109).

Figure 4 shows that 27.93% of incidences of lumbar spine pain were reported between days 12-14, 13.51% between days 21-23, and 17.12% between days 24-26%. All other days failed to record 10% of the total incidences.

Using a  $\chi^2$  test on the data ( $\chi^2 = 35.914$ ,  $df = 10$ ) the results were found to be significant ( $p < 0.001$ ). This shows that the null hypothesis that low-back pain is likely to occur with the same frequency anywhere in the cycle can be rejected.



## Analysis of Cervical Spine Pain

Figure 5 shows the frequency of incidence in relation to cervical spine pain alone occurring in each three-day time band starting from day one of the subject's menstrual cycle.

Figure 6 shows that 17.65% of incidences of cervical spine pain were reported between days 12-14, 13.51% between days 21-23 and 14.71% between days 24-26. At the start of the cycle during the menstrual phase, incidences of 11.78 % were reported for days 0-2 and 11.76% for days 3-5; this fell to less than 5% during days 6-8 only to rise again in the pre ovulatory phase. The number of reported incidences then fell in the early luteal phases but not as sharply as in the low-back pain group. They then rose to the secondary peak as the cycle approached days 24-26.

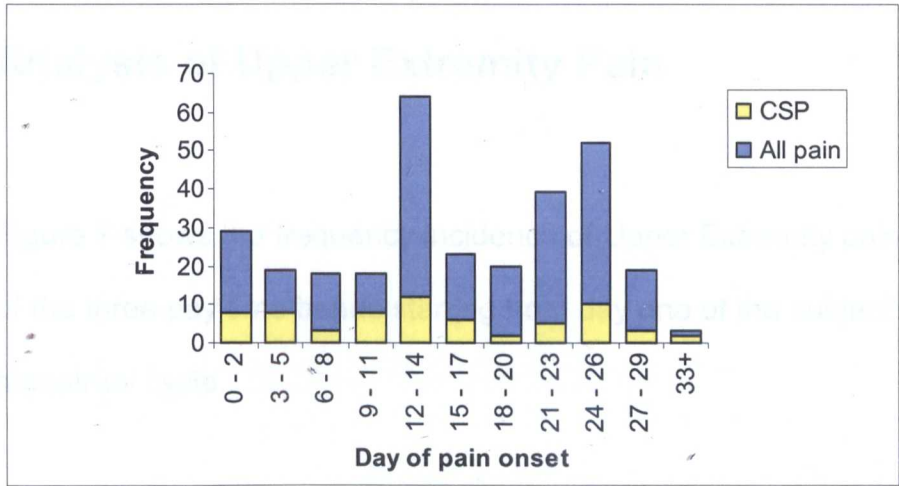


Figure 5: Incidence of "Cervical Spine Pain" as displayed with "All pain" in the menstrual cycle (n=67).

These results demonstrate that a higher frequency of incidences were reported around days 12-14, days 21 to 23 and days 24-26 .

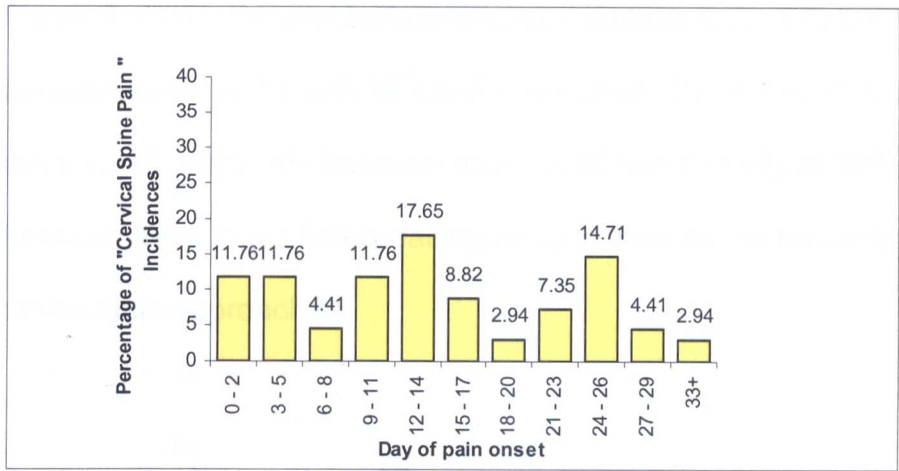


Figure 6: Percentage of “Cervical Spine Pain” incidence in the menstrual cycle (n=67)

Using a  $\chi^2$  test on the data ( $\chi^2 = 87.263$ ,  $df = 10$ ) the results were found to be significant ( $p < 0.001$ ). This shows that the null hypothesis that cervical pain is likely to occur with the same frequency anywhere in the cycle can be rejected.

## Analysis of Upper Extremity Pain

Figure 7 shows the frequency incidence of Upper Extremity pain in each of the three day time bands starting from day one of the subject’s menstrual cycle.

It shows that the incidence of pain reported arising from the upper extremities was not high amongst the subjects reporting pain. When it

was there it was found mainly in the second half of the cycle, peaking in frequency between days 12-14.

Figure 8 shows the greatest percentage of upper extremity pain was between days 12-14 with 36% being reported. This fell to 16% between days 15-17 and to 8% between days 18-20 rising briefly to 16% again between days 21-23 before falling away to zero as the menstrual phase of the cycle approached.

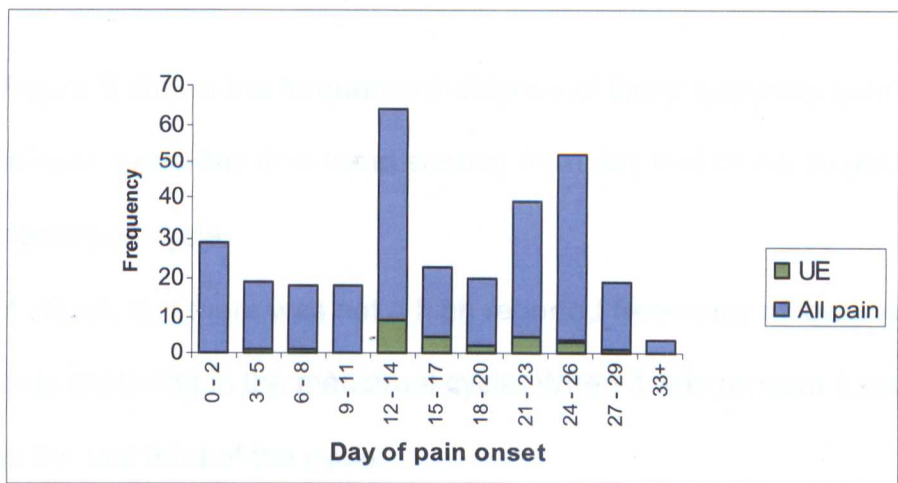


Figure 7: Incidence of “Upper Extremity Pain” as displayed with “All Pain” in the menstrual cycle (n=25).

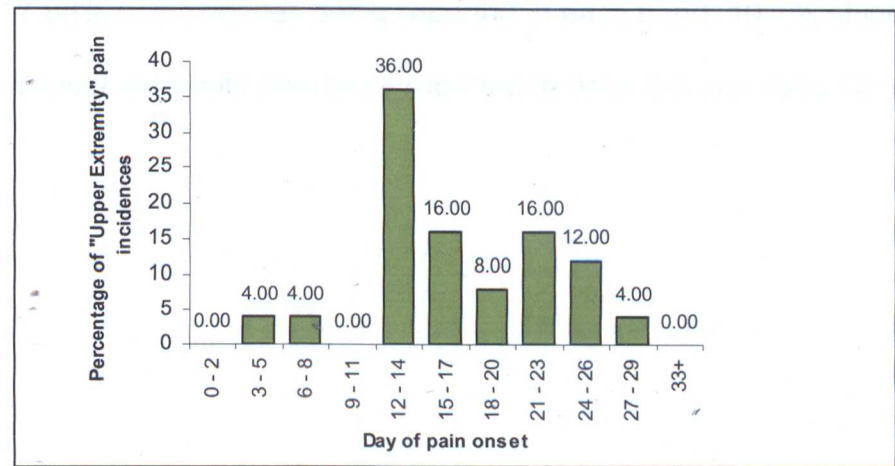


Figure 8: Percentage of “Upper Extremity Pain” incidence in the menstrual cycle (n=25).

Using a  $\chi^2$  test on the data ( $\chi^2 = 6.13$ ,  $df=10$ ) the results were found not to be significant ( $p>0.05$ ). This suggests that in the upper extremity, pain unlikely to occur at any one specific time in the cycle compared to any other time in the cycle. This null hypothesis cannot be rejected.

## **Analysis of Lower Extremity pain**

Figure 9 shows the frequency incidence of lower extremity pain occurring in each three-day time band starting from day one of the subject's menstrual cycle.

It shows that there was not a high reported frequency of lower extremity pain occurring in the menstrual cycle. When it was present it was mainly in the last third of the cycle.

Figure 10 shows that the greatest percentage of incidences of lower extremity pain were reported between days 21-23, 24-26, and 27-29 with 17.86% of incidences being reported in each band. 10.1% of incidences of lower extremity pain were reported in days 3-5 and days 12-14.

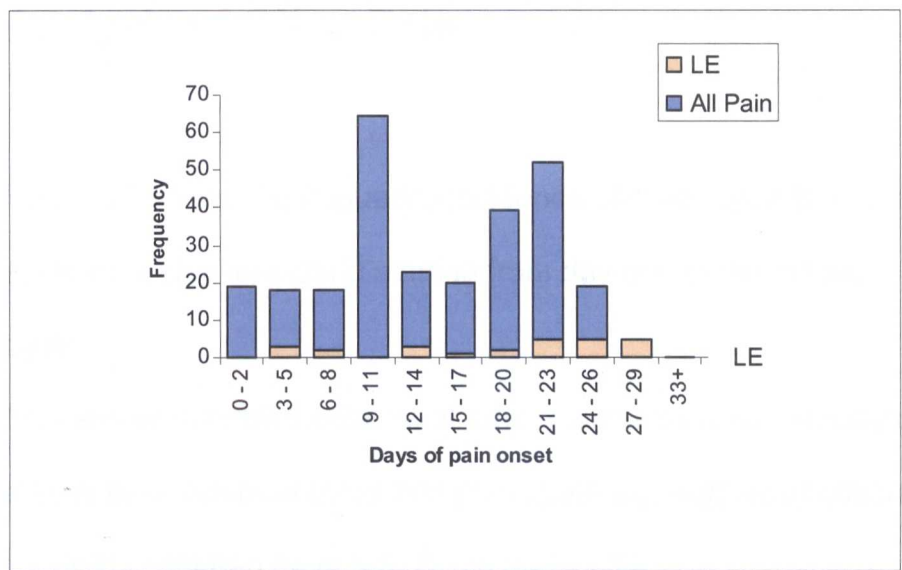


Figure 9: Incidence of “Lower Extremity Pain” as displayed with “All Pain” in the menstrual cycle (n=25).

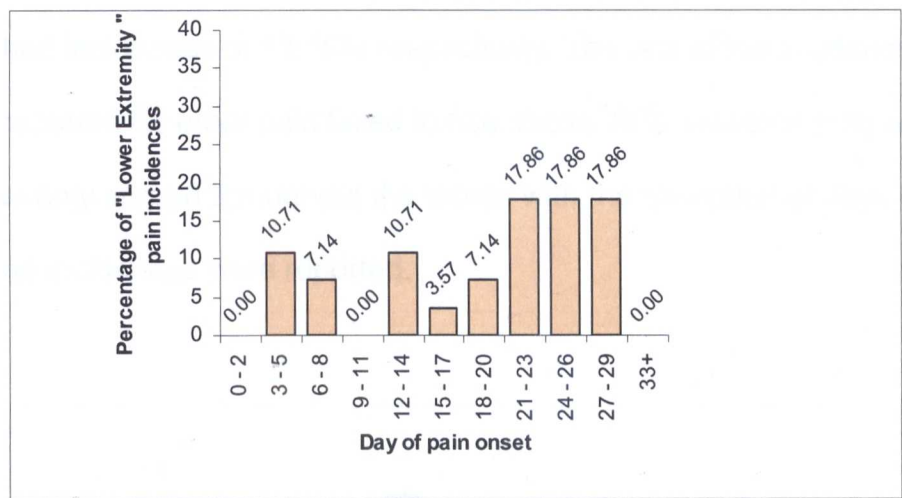


Figure 10: Percentage of lower extremity pain incidence in the menstrual cycle (n=25).

Using a  $\chi^2$  test on the data ( $\chi^2 = 5.56$ ,  $df = 10$ ) the results were found not to be significant ( $p > 0.05$ ). This suggests that as was the case with the upper extremity, pain in the lower extremity is unlikely to occur at any one specific time in the cycle compared to any other time in the cycle. The null hypothesis cannot be rejected



## Analysis of Other Pain

Figure 11 shows the frequency incidence of other pains being reported in each three-day time band starting from day one of the subject's menstrual cycle.

This shows that the incidence of other pains was nearly evenly distributed across the menstrual cycle with small peaks of frequency occurrence occurring between days 3-5, 24-26 and 27-29.

Figure 12 shows that the greatest percentage of incidences of other pain was reported between days 27-29 with 19.74%. Days 3-5 and days 24-26 had incidences of 13.16% respectively. The rest of the incidences being reported for other pain failed to rise above 10%; however they were evenly spread throughout the month with the exception of days 0-2 where no incidences were reported.

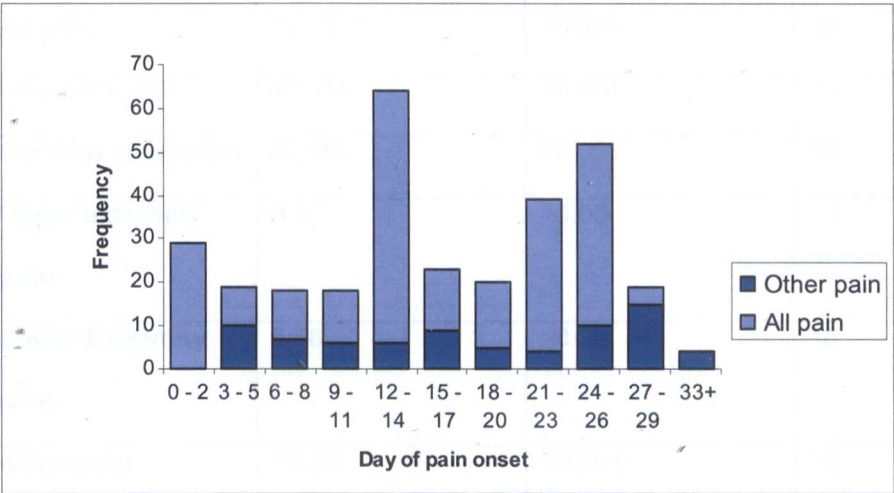


Figure 11: Incidence of "Other Pain" in the menstrual cycle as displayed with "All Pain" (n=74).

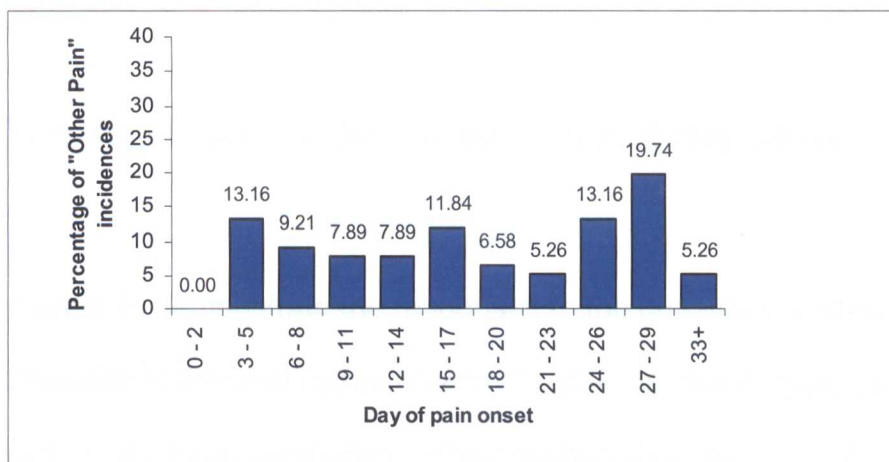


Figure 12: Percentage of "Other Pain" incidence in the menstrual cycle (n=74).

Using a  $\chi^2$  test on the data ( $\chi^2 = 58.291$ ,  $df = 10$ ) the results were found to be significant ( $p < 0.001$ ). This shows that the null hypothesis that other pain is likely to occur with the same frequency anywhere in the cycle can be rejected.

Table 2: The  $\chi^2$  and p values for site of pain

	$\chi^2$	p	df
All pain	111.018	<0.001	10
Low-back pain	35.914	<0.001	10
Cervical spine pain	87.263	<0.001	10
Upper extremity pain	6.13	>0.05	10
Lower Extremity pain	5.56	>0.05	10
Other pain	58.291	<0.001	10

## The Analysis of the Chronicity of the pain

In order to demonstrate the responses to the questions relating to the character of the pain in the menstrual cycle, bar charts were plotted for each of the three categories in the questionnaire relating to chronicity of pain, acute chronic and recurrent, as found in the results from the questionnaire.

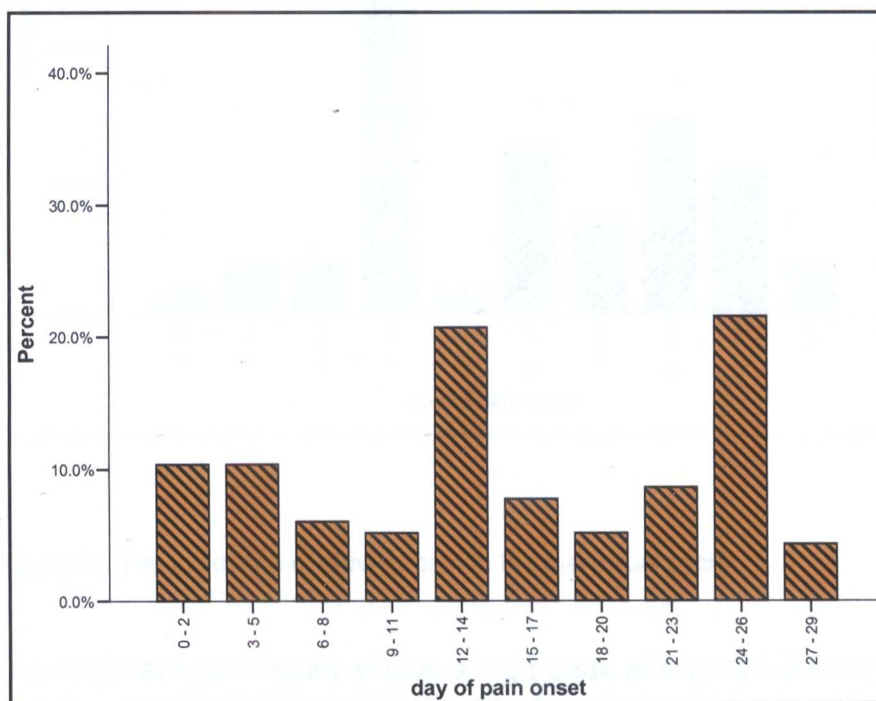


Figure 13: The Incidence of acute pain in the menstrual cycle

Figures 13, 14, and 15 show that there are specific patterns with regard to pain onset within the cycle and the chronicity of pain. Acute pain has two peaks one relating to the mid-cycle at days 12-14 and one to days



24-26, with incidences of 20% and 22% respectively. The other phases of the cycle did not have incidences that rose above 10%.

The chronic pain figure shows a peak only at days 12-14, but it was a high peak of nearly 40% incidence. The level of incidence in the rest of the cycle for chronic pain did not rise above 15%.

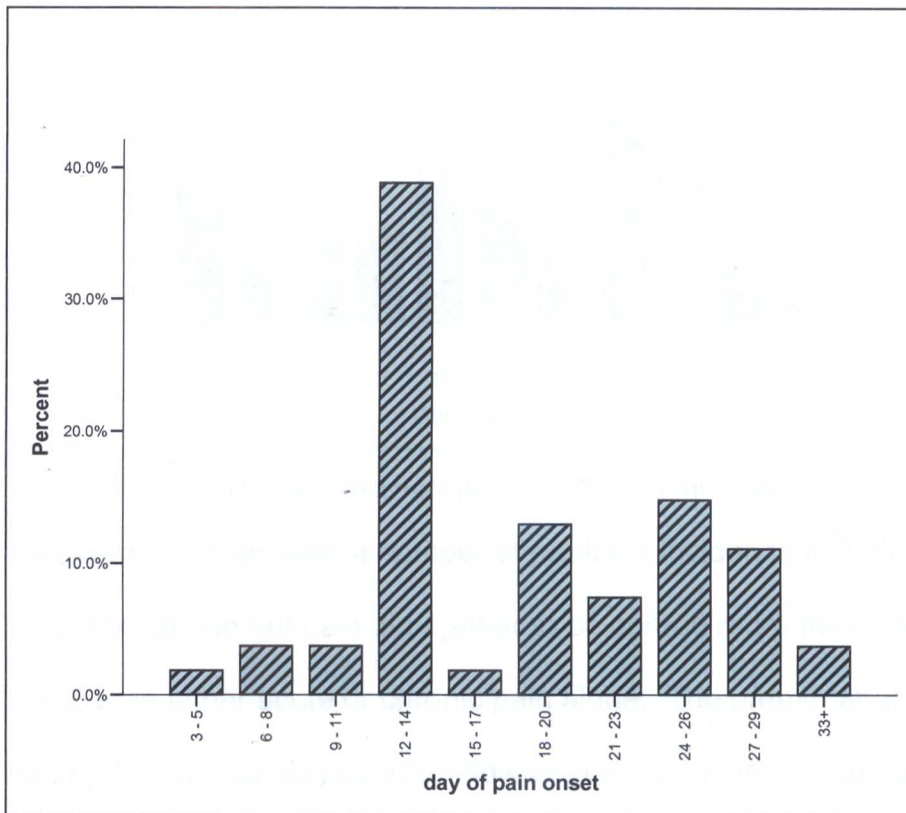


Figure 14: The incidence of chronic pain in the menstrual cycle

The recurrent pain figure shows a high peak at days 21-23 with an incidence of 18%, but also peaks at 0-2 days 12-14 days and 24-26 days with incidences between 10% and 15% for each peak.

Thus it appears that the ovulatory phase of the cycle occurring between days 12-14 showed a peak in incidence for each category of chronicity, but particularly in regard to chronic pain.

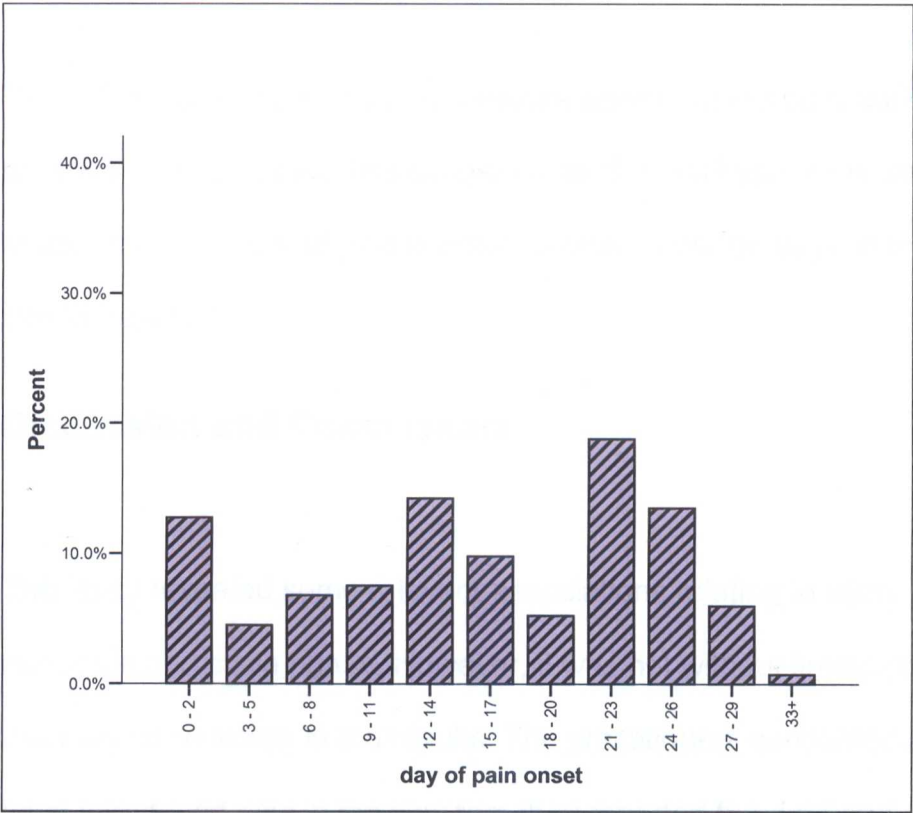


Figure 15: The incidence of recurrent pain in the menstrual cycle

Recurrent pain showed a number of peaks of incidence including the ovulatory phase but pain did appear to be spread more throughout the cycle than either acute or chronic pain alone. The premenstrual phase of the cycle between days 24-26 showed peaks of incidence for each category of pain.

Table 3: The  $\chi^2$  and p values for chronicity of the pain

	Chi <sup>2</sup>	p	df
Acute Pain	56.827	<0.001	10
Chronic Pain	88.579	<0.001	10
Recurrent Pain	40.570	<0.001	10

Using Chi<sup>2</sup> tests on the data, the results were found to be significant at  $p < 0.001$  for each case. This suggests that the null hypothesis which states that chronicity of pain is not associated with the days of the cycle can be rejected.

## **Discussion and Conclusions**

The study revealed some relevant associations relating to injury incidence and cycle phase. However, it was not without limitations and this may be reflected in the results. The practitioners concerned may have introduced bias in the way that they reported the data and completed the forms. Did they understand the point of the survey and could they have unwittingly introduced their bias into the results in order to agree or disagree with the hypothesis?

The design of the questionnaire was kept specifically brief and simple so as to isolate the research question. This may have produced a large group of "other pains".

A different design of the questionnaire might have separated this group into smaller categories and thus improved the category response groups by increasing them. It was interesting to learn from the study that the incidence in cervical spine pain and the incidence of low-back pain were different.

The results relating to chronicity of a pain showed that acute, chronic, and recurrent pains also have different patterns of presentation. The use of a visual analogue scale, for example, would have given greater objectivity to this response because the words acute and chronic are subjective, and not objective terms, which gives a potential imprecision to the responses received.

If cyclical female hormones have an influence on joint function through their effects on collagen or skeletal muscle for example, then the question is posed, "Why do different tissues respond at different times?" Is it because of the histological difference of the tissues in the cervical spine as opposed to the lumbar spine or pelvis, or are there receptors in different tissues which respond to specific hormones and which therefore are clinically significant only at specific times within the cycle?

Furthermore does this have an effect on the chronicity of pain in the cycle?

The literature review demonstrated that cyclic female hormones have an effect at a number of levels including pain thresholds, the changes in the circulating levels of cyclic female hormones act on the central nervous system and on pain transmission mechanisms, and can potentially influence athletic injuries.

The results of this study show there is a clear relation between pain onset in the menstrual cycle and the chronicity of that pain. Even the site of the pain may differ in each cycle phase.

Based on these results patients might be advised to avoid anything which might adversely challenge their musculo-skeletal systems such as carrying heavy loads or vigorous sports especially if they have a history of acute, recurrent or chronic musculo-skeletal pain, as the risk of injury might be higher at these times. They should certainly be advised to note when injuries occur in the future, and to ascertain if they are in fact occurring at specific times within the menstrual cycle. This would add weight to the advice for avoiding injuries that could become repetitive. The fact that female athletes injure themselves with greater frequency than their male counterparts has been well reported. Female athletes have a two to eight-fold higher incidence of ACL injury than their male counterparts (Toth and Cordasco, 2001), but the evidence required to advise female athletes to change their training or the timing of their events has not been sufficient to date. This study does suggest that ligamentous laxity during the normal menstrual cycle is another factor to be considered when understanding the puzzle of the timing of female athletic injuries. However, it is not simply ligaments alone that contribute to joint laxity. Skeletal muscle changes and the potential for skeletal muscle fatigue might also alter joint laxity if muscle strength varies at different times in the menstrual cycle or during pregnancy or after the menopause.

The next part of the thesis reviews the literature relating to the sex hormones and the menstrual cycle as well as their effects on tissues

remote from the reproductive system; it is proposed to investigate whether any such changes have a global or simply a local effect

Using the hyperextensometer, laxity was measured on specific days within the cycle. Blood measurements for the hormones oestrogen, progesterone, and relaxin were determined to ascertain the potential link between hormone levels and joint laxity.

# ***Chapter Two:***

# ***Introduction to the***

# ***experimental studies***

## **Introduction to the experimental studies**

The pilot study demonstrated that there are potential peaks of incidence of acute musculo-skeletal pain in relation to the menstrual cycle. There are many possible explanations for this pattern, and the clear association between the calendar and the onset of pain was thought to be worthy of further study.

Hormones are capable of affecting the physiology of many body tissues so long as the tissues concerned have receptors to the hormones concerned.

The factors that control movement around a synovial joint include skeletal muscle tissue with all of the associated stretch receptors and Golgi tendon organs, the tendons themselves, the joint capsule, the ligaments, the articular cartilage, and the neuronal pathways responsible for proprioception, as well as the brain and higher centres.

The receptors for the sex hormones are located in the nuclei of the target tissues. Is the sensitivity of these tissues to the sex hormones capable of change during the menstrual cycle and thus is the joint laxity likewise capable of change? Muscular and ligamentous tissues in particular have an important role to play in protecting the joints from overstrain and dislocation at the end of their range. Is there somehow a weakening



effect of the sex hormones on the tissues concerned and was this in some way responsible for the pain patterns that emerged during the pilot study?

In order to investigate the effects of these sex hormones on joint mobility, a series of studies was designed to investigate the mobility of a single synovial joint at various stages within a menstruating woman's life, to ascertain the relationship between these stages and potential changes in mobility.

A single synovial joint was chosen because if it changed, that change would potentially be representative of a global change on all body tissues in relation to the circulating sex hormones, and not just a change within the pelvis.

The equipment used was the Joint Hyperextensometer first designed by Jobbins, Bird and Wright in Leeds in 1979. The advantages of this equipment are that it is light and portable, and according to the authors (Jobbins , Bird et al., 1979) it is an accurate and reproducible way of measuring the range of movement into hyperextension at the carpo-metacarpal joint of the index finger of the dominant hand. It is a non-invasive procedure that takes less than a minute to use and so was felt suitable for evaluation with regard to future use as the instrument of choice in this series of studies.

## The Hyperextensometer

The Hyperextensometer is a simple piece of apparatus comprising of a carrier for the index finger of the dominant hand, which is mounted on a shaft supported in rolling elements which are themselves mounted in housing. The whole assembly is fastened to a base plate. Velcro tape is used to secure the first interphalangeal joint of the index finger of the dominant hand to the carrier in such a way as to align the rotational axis of the metacarpo-phalangeal (MCP) joint parallel to the axis of the operating shaft.

Rotation of the joint was caused by manually rotating a knurled knob which was co-axial with the operating shaft, and which drives the operating shaft via a clutch mechanism.

This clutch mechanism is of the spring loaded ball détente type and can be pre-set to slip at a given value of torque. It is capable of applying pre-set torque varying between 2.0 and 7.0 kg/cm (0.19 and 0.68 Nm). The amplitude of rotation of the index finger at the moment of slip was indicated by a pointer fixed to the operating shaft and in close proximity to a protractor that was fixed to the housing as shown in Figure 16 below.

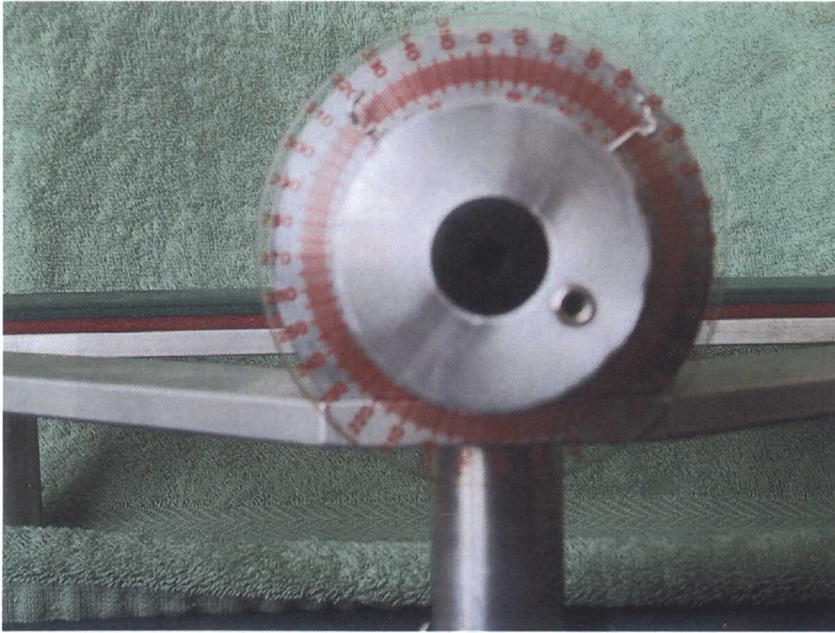


Figure 16: The Hyperextensometer from the front

Operation of the Hyperextensometer is straightforward. The subject is made comfortable with the forearm and hand of the dominant side supported by the base plate. The axis of rotation of the meta-carpo-phalangeal ( MCP) joint of the index finger is aligned parallel to the axis of the operating shaft by the researcher looking from above and from the side to ensure that the internal angles are 90 degrees and that the axes of rotation of the operating shaft and the axis of rotation of the MCP are correct.

The finger is strapped to the carrier such that there is no movement possible from the intermediate phalangeal joint.

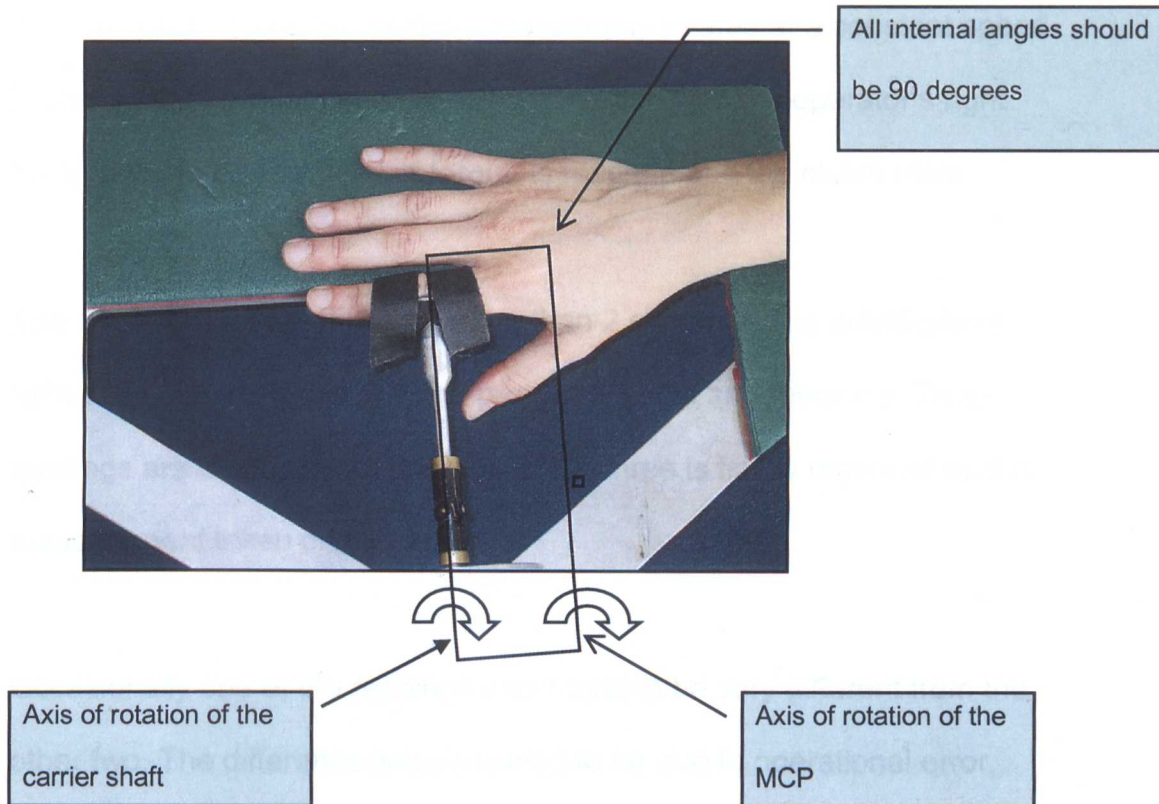


Figure 17: Alignment of the subject's hand on the Hyperextensometer

The operator places the flat of his left hand firmly onto the dorsum of the subject's hand so as to ensure that there should be no movement at the wrist joint during the measurement. It is essential that the forearm of the subject remains in contact with the base plate, and that the internal angles of 90 degrees as shown in the photograph above, is maintained at all times. This procedure is designed to obtain the maximum possible consistency of the readings and to eliminate a possible source of error.

The indicating pointers are zeroed, and the knob is rotated counter-clockwise until a click is heard which resets the clutch mechanism. The apparatus is now set for measuring. The angles mentioned before are

checked once again, before the knob is firmly rotated at a constant speed in one swift movement in a clockwise direction with the operator's right hand until the pre-set level of torque is reached and the clutch slips.

The whole procedure takes no more than 2 seconds. The amplitude of rotation is then measured on the protractor scale and recorded. Three readings are taken and an average of the three is finally recorded as the measurement taken on the day.

Occasionally one of the readings was found to be very different from the other two. The difference was assumed to be due to operational error, and if this was the case the readings were repeated in groups of three until three consecutive readings enabled a consistent average to be recorded. This was not a common occurrence, and considering the many hundreds of readings obtained from the apparatus it was not thought to influence the accuracy of the overall results.

## **Validation of the Hyperextensometer**

Preliminary studies showed the following to be critical to obtaining reliable data:-

The subject had to be sitting and relaxed

The elbow had to be bent to 90° and supported

The forearm had to be parallel with the ground

The axis of the movement of the MCP joint of the index finger had to coincide with the axis of the machine although minor variations in position were not critical.

The firm pressure on the rest of the hand and the wrist prevented any extraneous movement interfering with the measurement of the MCP joint alone.

In practice it was found that repeat readings in a single individual became consistent with adherence to these precautions.

In the original paper by Jobbins et al., (1979) it was stated that a torque on the clutch mechanism of 2.6 kg.cm ( 0.26 Nm) caused both extremes of the range of movement observed in a large population studied to fall within the recording range of the machine. Hence for joint laxity studies the machine was set at this value.

Jobbins et al., (1979) studied the correlation of the Hyperextensometer both with the Beighton Ligamentous Laxity Scoring system (LLS), a standard rheumatology test method in common usage, and a more complex global score of joint laxity devised by themselves and calculated by summing the range of movement measured by goniometer of all the joints in the body. In a population of 54 individuals, some selected to demonstrate extreme joint laxity, both correlations were significant, for the Beighton scale ( $p < 0.001$ ), and for the global index ( $p < 0.001$ ). That confirmed that comparisons can be made with the clinical scoring system

in common use and suggests that, at least in the population tested, laxity at the MCP joint of the index finger reflects joint laxity in the rest of the body.

***Chapter Three:***  
***The Menstrual Cycle***  
***Study***



## Introduction

The human menstrual cycle is a series of predictable biochemical and physical events that occur in sexually mature females approximately every twenty eight days. The changes in the sex organs and other tissues are governed by the production of sex hormones, the most significant being oestrogen, progesterone and relaxin. These hormones have profound effects on all body tissues including connective tissues. This thesis examines the effects of cyclic sex hormones in women. The first experiment investigates the potential links between the events in the normal menstrual cycle and stability and laxity in a synovial joint. The underlying premise is that if women are found to hurt themselves during the menstrual cycle, the incidence of injury is related to the phases of the cycle and is potentially related to laxity in synovial joints. The logical place therefore to begin this investigation is with a review of the events that happen within the menstrual cycle and with a review of the evidence that might exist linking these events and the factors that control movement at synovial joints.

## Chemistry Biosynthesis and Metabolism of Oestrogens

There are three naturally occurring oestrogens in significant quantities in the plasma of human females,  $\beta$  oestradiol (E2), oestrone (E1), and oestriol. The principal oestrogen secreted by the granulosa cells of the ovarian follicles is  $\beta$  oestradiol. Small amounts of oestrone are also secreted, but most of this is formed in the peripheral tissues from androgens secreted by the adrenal cortices. A small amount is secreted by the thecal cells of the corpus luteum.

The oestrogenic potency of  $\beta$  oestradiol is 12 times that of oestrone and 80 times that of oestriol (Guyton and Hall, 1996). For this reason  $\beta$  oestradiol is considered to be the major oestrogen and therefore the number of its receptors to be found in tissues is an indicator of how those tissues are affected in their function by the presence of the hormone.

Oestradiol, oestrone and oestriol are metabolised in the liver, and all these compounds along with other metabolites are excreted in the urine. Appreciable amounts are secreted in the bile and reabsorbed back into the bloodstream in the enterohepatic circulation.

Figure 18 and Table 4 show the concentration of the hormones in the plasma during the menstrual cycle. Almost all of the oestrogen comes from the ovary, and there are two peaks in the volume of its production: one is just before ovulation and one is during the mid-luteal phase. After

menopause, oestrogen secretion declines to very low levels. The volume of progesterone is highest in the mid-luteal phase of the cycle.

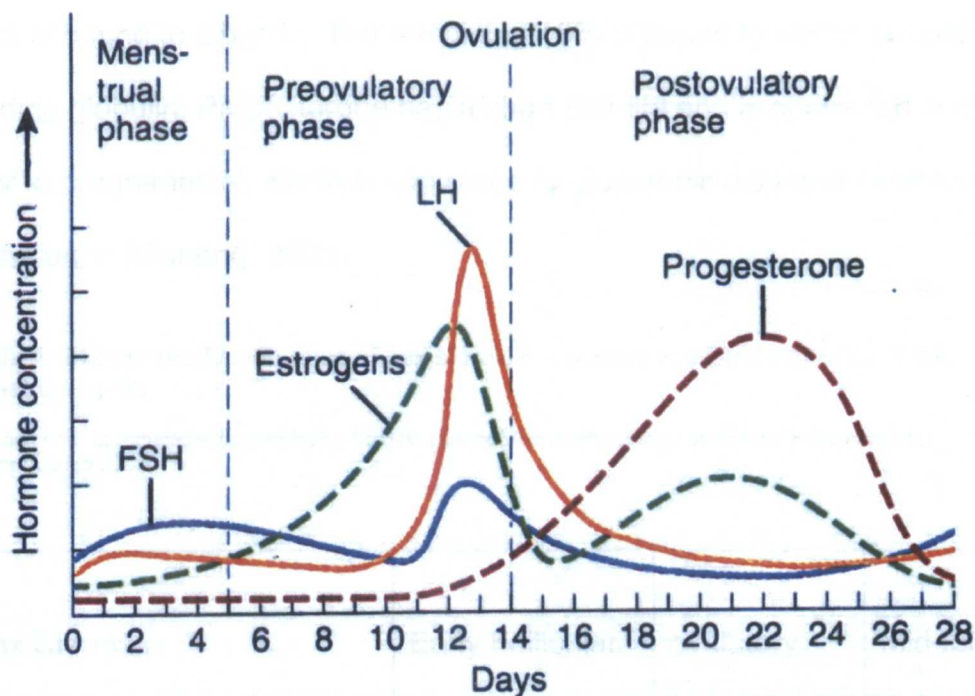


Figure 18: The hormones of the menstrual cycle.

From Tortura and Grabowski page 1034 9th Edition Published by John Wiley and sons .2000

## Chemistry, Biosynthesis, and Metabolism of Progesterone

Progesterone is secreted by the corpus luteum, the placenta, and (in small amounts) the Graafian follicle of the ovary. It is an important intermediate in steroid biosynthesis in all tissues that secrete steroid hormones, and small amounts apparently enter the circulation from the testes and from the adrenal cortex.

The hormone  $\alpha$  hydroxyl progesterone is secreted along with oestrogens from the ovarian follicle and its secretion is in parallel with that of  $\beta$  oestradiol. Two percent of the circulating progesterone is free, whereas 80% is bound to albumin. The remaining 18% is bound to corticosteroid binding globulin. Progesterone has a short half-life and is converted in the liver to pregnanediol, which is converted to glucuronic acid and excreted in the urine (Ganong, 2003).

Table 4: 24 hour production rates of sex steroids in women in different phases of the menstrual cycle.

Modified and reproduced with permission from Review of Medical Physiology by William F Ganong 21st Edition; Lange 2003

Sex Steroids	Early Follicular	Pre-ovulatory	Mid-luteal
Progesterone (mg)	1.0	4.	25
17HydroxyProgesterone(Mg)	0.5	4	4
Androstenedione (Mg)	2.6	4.7	3.4
Testosterone ( $\mu$ g)	144	171	126
Oestrone ( $\mu$ g)	50	350	250
Oestradiol( $\mu$ g)	36	380	250

Oestrogen and progesterone, like all steroids, are small hydrophobic molecules. Thus they diffuse freely through the plasma membrane of all cells. However, in target cells, which are cells that change their gene

expression in response to a hormone, such as those of the endometrium, they become tightly bound to oestrogen and progesterone receptors. The complex of receptor and its hormone moves to the nucleus, and there it binds to a specific hormone response element. These response elements are specific sequences of DNA, in the promoters of certain genes that are needed to turn genes on and off. Thus the complex of the hormone and its receptor forms a transcription factor which is a protein needed to initiate the transcription of a gene (Kimball, 2004). Receptors for progesterone are not as widespread as those of oestrogens, and are usually only to be found in very specific sites.

The presence of progesterone receptors located in the nuclei of smooth muscle cells in the round ligament was demonstrated in 1993 (Smith et al., 1993). The authors looked at samples of the round ligament in pre- and post-menopausal women and analysed them histochemically for oestrogen and progesterone receptors. They found that the round ligament was a specific target organ for these hormones. It is unlikely therefore that this hormone is going to be actively involved in changes in peripheral joint laxity within the menstrual cycle by an action on connective tissue alone. It might well be a partial effect on skeletal muscle strength but there is a lack of evidence to support this view. The changes in laxity are more likely to be due to the effects of oestrogen and relaxin on these tissues.

## **Chemistry Biosynthesis and Metabolism of Relaxin**

Produced by a range of species, relaxin is a reproductive hormone with a role in pregnancy (Weiss 1991). The hormone regulates the growth and remodelling of reproductive tissues during late pregnancy. For instance in the pig, rat and guinea pig, the hormone helps loosen the cervix during parturition (Sherwood 1994). In humans, however, the hormone is at its highest concentration early in pregnancy, leading researchers to believe that it is involved in the implantation of the blastocyst (Schwabe and Bullesbach, 1990; Schwabe and Bullesbach, 1994; Johnson et al., 1991; Johnson et al., 1994).

Outside of pregnancy, relaxin is also secreted during the formation of new blood vessels, during wound healing, and can contribute towards counteracting the effects of congestive heart failure. These and other functions mean that relaxin – when more is understood about the hormone – could be an excellent candidate as a drug for a whole range of illnesses and disease (Bani 1997).

Relaxin is a peptide with a molecular weight of approximately 6,000 Daltons, it consists of two dissimilar A and B chains linked by disulphide bridges. It is an analogue of insulin that shows no cross reactivity to insulin receptors.

In humans two molecular forms of relaxin have been identified, with high sequence homologues designated H1 and H2 relaxin, and encoded by two distinct genes. Of these H2 relaxin corresponds to the circulating relaxin produced by the ovary. The H1 form of relaxin seems to be produced only by the decidua (Bani, 1997).

Relaxin is produced by a range of mammalian species. During pregnancy relaxin is thought to determine not only the time of onset of labour but also its subsequent progression (Johnson et al., 1993). Furthermore it has been shown to be important in the normal growth of the uterus and endometrium in primates and rodents, and may have a similar role in women.

Relaxin has been shown to be produced in the luteal phase of non-pregnant women every cycle as shown in figure 19 , although the reason for this is unsure. It may have an effect the softening and opening the cervix which allows for the monthly menstrual flow (Johnson et al., 1993). The factors that regulate the levels of circulating relaxin during the menstrual cycle and during pregnancy are uncertain, although animal and some human data suggest that in pregnancy at least the levels are determined in the cycle of conception.

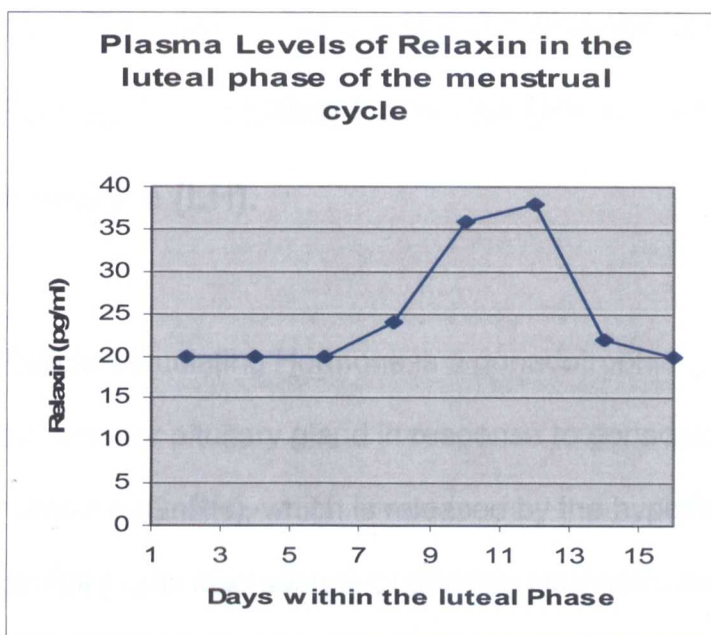


Figure 19: The geometric means of the plasma levels of relaxin during the luteal phase of the menstrual cycle in nine normal volunteers.

From Relationship between ovarian steroids, gonadotrophins, and Relaxin during the menstrual cycle.

Johnson MR et al., .Acta Endocrinologica 1993 129: 121-5

There is further evidence to suggest that there is a strong positive relationship between the circulating levels of relaxin and those of oestrogen as E2 (Johnson et al., 1993). This implies that either the synthesis of E2 and relaxin is regulated by a common factor, or that E2 rather than LH regulates relaxin synthesis during the menstrual cycle. This work has been confirmed by other researchers (Samuel et al., 1996). The degradation pathway for relaxin is unclear. It is probably mediated through the liver and excreted via the bile and /or the urine (Ganong 2003).



## **The Chemistry, Biosynthesis, Metabolism and actions of Follicle Stimulating Hormone (FSH) and Lutenising Hormone (LH).**

Follicle Stimulating Hormone is a gonadotrophic glycoprotein secreted by the anterior pituitary gland in response to gonadotrophin-releasing hormone (GnRH), which is released by the hypothalamus. The same pituitary cells also secrete Lutenising Hormone, another gonadotrophin. Both FSH and LH are composed of alpha and beta subunits. The specific beta subunit confers the unique biological activity. Both FSH and LH bind to receptors in the testis and ovary and regulate gonad function by promoting sex steroid production and gametogenesis (Jabboeur, 2002).

In females, LH stimulates oestrogen and progesterone production from the ovary. A surge of LH in the mid-menstrual cycle is responsible for ovulation, and continued LH secretion subsequently stimulates the corpus luteum to produce progesterone. Development of the ovarian follicle is largely under control of FSH, and the secretion of oestrogen from this follicle is dependent on both FSH and LH (Jabboeur 2002).

Despite an extensive search of resources on the current Medline database, the National Library of Medicine database and OVID online, evidence has yet to be found showing receptors in collagen tissues or skeletal muscle for FSH or LH. The effect of these hormones appears to

be confined to the production of oestrogen and progesterone and it is these hormones which, as stated above, can have an effect on joints and skeletal muscles.

The degradation pathway for LH and FSH is again unclear. It is probably mediated through the liver and excreted via the bile and the urine.

### **The role of Oestrogen, Progesterone and Relaxin in the normal menstrual cycle**

The physiological effects of oestrogen are widespread. It is a hormone responsible for the secondary sexual characteristics in females, and it influences the production of the cervical mucus and the structure of the vaginal epithelium. During the menstrual cycle oestrogen causes the proliferation of the uterine endometrium. It inhibits FSH and encourages fluid retention.

The effects of progesterone are mainly evident in the second half of the cycle. It causes secretory changes in the lining of the uterus, where the endometrium develops tortuous glands and an enriched blood supply in anticipation of a fertilised ovum. These changes occur only in an endometrium already primed by a high level of oestrogen.

Relaxin is found only in the normal menstrual cycle during the late luteal phase. Its action has been deemed to be on the cervix where it changes the composition of the collagen fibrils and helps to ripen the mucous plug and allows the cervix to open just as it does during labour (Sherwood, 1994). The actions of relaxin outside of the genital tract are numerous and will be dealt with later.

## **Oestrogen and Skeletal Muscle**

That there are nuclear receptors in skeletal muscle for male and female sex hormones is well established. Oestrogens exert their action via specific protein receptors that are members of the steroid /thyroid receptor super family of transcription regulation factors (Taylor and Al-Azzawi, 2000).

By 1980 androgen receptors were detected in the quadriceps muscles of adult male and female rats (Michel and Baulieu, 1980). However, despite using a variety of techniques for separating and measuring hormone receptor complexes, at that time, oestrogen receptors had not been found. The androgen-binding sites were of relatively low concentration. However, the typical binding specificity of the androgen receptor and the regulatory effects of the androgens on their own receptor supported the possibility that some effects of androgens on skeletal muscles may have been initiated directly at the cellular level through this receptor.

By 1982 the presence of oestrogen receptors in the cytosol of rat skeletal muscle had been established (Dahlberg, 1982). Later research showed that the mechanism of action of steroid hormones in target tissues included the binding of the steroid molecule to specific receptors in the cytoplasm (Gustafsson et al., 1984). Steroid receptors were therefore regarded as mediators of hormone actions. The number of such receptors in tissues reflects their hormone sensitivity, and the receptor levels are indicative of the relative potential for the direct hormonal action of the tissue in question. Using synthetic ligands and a charcoal absorption assay, the presence of specific androgen, glucocorticoid and oestrogen (in rabbits only) receptors was demonstrated in human and rabbit skeletal muscle cytosol. These tissues were therefore regarded as targets for these hormones (Dahlberg, 1982).

In 1984 research was undertaken into hormone receptors in human spinal muscles of patients diagnosed with scoliosis (Saato et al., 1984). Twenty patients of both sexes, at different ages, and with different aetiological reasons for the condition were investigated. Androgen and glucocorticoid receptors were found but surprisingly no oestrogen receptors.

Using a more sophisticated method of analysis it was found that steroid receptor complexes existed for each specific steroid. The concentration of androgen and oestrogen receptors was of the same magnitude, whereas

the corresponding value for the glucocorticoid receptor was about ten-fold higher (Gustafsson et al., 1984). This is not surprising considering the physiological effects of the glucocorticoids on skeletal muscle.

In 1986 and 1987 two papers were published reporting the presence and distribution of oestrogen receptors in the temporo-mandibular joints ( TMJ) of baboons (Aufdemorte et al., 1986; Milam et al., 1987). It was found that the TMJ complex contains numerous cells with receptors for oestrogen. The receptors were found in the articular surfaces of the condyles, articular disc, and capsule in the females but not in the males. Likewise the muscles of mastication contained relatively few receptors. As a result the researchers postulated that the role for the sex steroids was one of maintenance and repair in the joint rather than a specific effect on the muscles of mastication acting over the jaw. Why this should be found in females rather than males, however, is still to be understood. Oestrogen receptors have been found in a diversity of muscle sites. The diaphragm, laryngeal muscles, pelvic floor muscles, urogenital ligaments and the myometrium all contain receptors, but not the rectus abdominus muscle. Again the reasons for this are still unclear (Bechet et al., 1986; Smith et al., 1990; Mann et al., 1995; Wu et al., 2003).

The latest work suggests the presence of two different oestrogen receptors in human tissue; these are oestrogen receptor alpha and oestrogen receptor beta (Lemoine et al., 2003). Both types of mRNA

have been found in mouse, rat, and bovine as well as human tissue samples (Wu et al., 2003). The expression of oestrogen receptor mRNA alpha was 180 times higher than that of oestrogen receptor mRNA beta.

In the ovary, oestrogen receptor beta was present in multiple cell types including granulosa cells in small, medium, and large follicles, thecae and corpora lutea, whereas oestrogen receptor alpha was weakly expressed in the nuclei of granulosa cells, but not in the theca or corpora lutea. In the endometrium both oestrogen receptor alpha and oestrogen receptor beta were observed in the nuclei of epithelial cells and in the nuclei of the stromal cells, but significantly oestrogen receptor beta was absent from the endometrial glandular epithelia. Oestrogen receptor beta cells were found in male urogenital tissues, and in most areas of the brains of both sexes (Taylor and Al-Azzawi , 2000).

It can be concluded, therefore, that the almost ubiquitous immuno-histochemical localisation of oestrogen receptor beta indicates that these receptors play a major role in the mediation of oestrogen's action outside of the musculo-skeletal system, whereas oestrogen receptor alpha seems to predominate within the musculo-skeletal system alone. The disappearance of these receptors, therefore, might have an important role to play in muscle strength and thus joint mobility after the menopause.

## **The effects of Oestrogen on muscle function**

Oestrogen affects muscle function in many ways. It has been shown to promote growth and accelerate skeletal development (Benson et al., 1989, Schiessl et al., 1998). Concentrations of oestrogen receptors in rabbits have been found to be higher in slow twitch fibres compared to fast twitch fibres (Gustafsson et al., 1984).

The structure of the oestrogen molecule has a very specific effect on growing muscle cells. This again must be a function of the oestrogen receptor types. Oestrone (a metabolite of oestradiol but possessing less biological activity) has been shown to induce a significant increase in myoblast growth, whereas beta oestradiol had no such effect (Kahlert et al., 1997).

The effects of beta oestradiol on the tension and fatigue responses of single fibre or fibre bundles prepared from frog skeletal muscles have been investigated (Hatae, 2001). The administration of the hormone caused a transient potentiation of tetanus tension by field stimulation at every minute.

Fatigue was produced by repeated tetanic stimulation every second until tension declined to approximately 40% of the initial level. Fibres were then allowed to recover. With the presence of beta oestradiol the time to

recover was less than for a control group. The presence of alpha oestradiol (an isomer) made no difference, suggesting the existence of very specific receptors in frog muscle (Hatae, 2001). These are most likely to be the beta receptors described above.

The effect of endurance training on transcripts of oestrogen receptor alpha in rat skeletal muscle has also been investigated (Lemoine et al., 2002). The results demonstrated that 7 weeks of endurance training increased the level of ER alpha receptors. This increase was greater in females compared to control groups.

Specific physiological variables and skeletal muscle functions may be capable of change along with menstrual cycle phases (Becker et al., 1982). However, the exact influence of the menstrual cycle phase on exercise performance, muscle strength, and fatigue is unclear, despite correlation between muscle strength and the various phases of the menstrual cycle having been the subject of many researches (Bruce et al., 1992; Phillips et al., 1992 a; Phillips et al., 1992 b; Phillips et al., 1993; Phillips et al., 1995; Phillips et al., 1996; Skelton et al., 1999; Janse de Jonge et al., 2001).

It is probable that this lack of clarity might be due to the variation of methods (muscle strength measure and assessment of cycle phase) and



variations of oestrogen levels in different subjects at the same phase of the menstrual cycle (Landgren et al., 1980; Bassey et al., 1995).

In her doctorate thesis, Onambele (2002) suggested that there can be a negative correlation, no correlation, or a positive correlation, between the menstrual cycle phase, the hormones and muscle strength.

*Negative correlation between normal menstrual cycle phase, oestrogen, and skeletal muscle force*

A group of studies in normally menstruating subjects report that muscle force increases when the oestrogen level is low. One study using tests such as handgrip and the standing long jump indicated that performance was significantly greater during the menstrual phase compared to the follicular and luteal phases (Davies et al., 1991).

*No correlation between normal menstruation cycle phase, oestrogen, and skeletal muscle force.*

Two studies suggest that muscle function, and muscle strength are independent of the phases of the menstrual cycle. One study suggested that the active woman with a normal menstrual cycle experienced no significant changes in maximum knee flexion and extension or endurance, as a result of cycle phase, (Rice, 1988; Janse de Jonge et al., 2001)

It has even been suggested that the reports of difference in muscle force with the menstrual cycle might be due to motivational factors rather than ability (Allen and Bailey, 1982). A study looking at the effects of motivation and cycle phase on the handgrip strength of a group of young women showed no significant differences either between groups (motivated or normal) or between the various phases of the cycle.

Friden et al., (2003) found that there was no significant variation in muscle strength and muscle endurance during the menstrual cycle. They used a serum measure for cycle phases and the LH surge was detected in the subject's urine (Friden et al., 2003). The self-criticism of this paper is interesting as the authors were careful to note this was a study of moderately healthy university students and not of highly trained athletes. It is the author's opinion that this is an important statement as the findings are more likely to relate to women in general and how and when they undergo injury than specifically to female athletes.

*Positive correlation between normal menstrual cycle phase, oestrogen, and muscle force*

A review of these and other studies reports a rapid muscle response to changes in oestrogen (Phillips et al., 1995). Some studies found that at mid-cycle, when the oestrogen levels are highest, muscle strength is greatest (Phillips et al., 1993; Phillips et al., 1995; Phillips et al., 1996;

Reise et al., 1995). Other studies report that the greatest difference in strength is seen between the luteal and ovulatory phases of the cycle, which is different from what would be expected from a direct oestrogen effect, whereby the greatest strength difference would be between the follicular and ovulatory phases (Sarwar et al., 1996).

In summary, the effects of oestrogen hormones on skeletal muscles are profound, and the greater the number of receptors the greater the possibility of the direct action of the hormone on the muscles concerned, especially if they are slow twitch fibres as are often found in postural muscles. It is the effects of the receptors to the various hormones that can contribute towards the strength or weakness of individual muscles.

Likewise if there are receptors in ligaments or other collagenous structures, it is important to assess the effects of hormone interactions via these receptors and the effects they might be capable of producing if a greater understanding of mobility changes in relation to hormones is to take place.

## **Oestrogen, Relaxin and Connective Tissues**

The effects of relaxin on the inter-pubic ligaments was first demonstrated more than 70 years ago (Hisaw, 1926).

All connective tissue remodelling occurs through a continuous cycle of protein synthesis and degradation (Dahlberget al., 1994; Foos et al., 2001). In this process, old or damaged structures are degraded and replaced with newly synthesized molecules. The balance between the degradative and biosynthetic arms of the process is controlled by the relative activities of matrix metallo proteinases and tissue inhibitors of metallo proteinases (Foos et al., 2001). The expression of some of these proteins is regulated by steroid hormones (Wahl et al., 1997; Rajabi et al., 1991; Matrisian, 1994). Oestrogen dependent collagenase production and progesterone dependent inhibition of collagenase have been observed in the pubic ligaments of pigs (Wahl et al., 1997). Furthermore, increasing the concentration of oestrogen in an ACL tissue culture model resulted in decreased fibroblast and pro-collagen production (Liu et al., 1997).

The effect is synergistic with the ovarian steroids and is likely mediated through the induction by relaxin of collagen remodelling. The exact method of action on collagen metabolism is still unclear; similar in vitro studies on human fibroblasts showed that relaxin promotes collagen secretion on one hand (Wiqvist et al., 1984) whereas it causes a decrease in collagen production and an increase in collagenase synthesis and collagen breakdown on the other (Unemori et al., 1990). Possibly, these conflicting results depend on the different responsiveness to relaxin of fibroblasts coming from different anatomical sites. These findings raise

the possibility that relaxin acts through the interplay between synthesis and demolition of collagen. Sherwood, (1994), in a major review of relaxin, thought that this hormone may have an outstanding role at parturition by inducing the metabolic changes in the connective tissues of the cervix and the inter-pubic ligament needed for collagen remodelling. In some species there is a relaxin surge in the pre-partum phase which is held responsible for successful dilation of the birth canal. However, because there is no relaxin in pre-labour humans, it has been suggested that local relaxin produced by the deciduas and the placenta and / or increased sensitivity by the target cells to relaxin ( when it appears) may be involved in these changes (Bryant-Greenwood and Schwabe, 1994).

The discovery of relaxin in the normal menstrual cycles (Johnson et al., 1993; Sherwood,1994), and thus its ability to induce collagen remodelling in situations other than during pregnancy suggests relaxin can potentially have a global effect rather than just a local effect on collagen (Bryant-Greenwood, 1982). Its therapeutic use for the induction of cervical ripening (MacLennan, 1991) may be important in assisting in cases of delayed labour.

The use of relaxin in the treatment of skin disease is also interesting but has now fallen out of favour (Casten and Boucek, 1958). Another research group in 2001 found that the use of recombinant relaxin in the

treatment of systemic sclerosis (or scleroderma) was ineffective (Gavino and Furst, 2001).

Relaxin has also been shown to influence renal vasodilatation, increase vascular endothelial growth factor expression and angiogenesis, promote dilation of blood vessels, and inhibit release of histamine (Bani, 1997).

Relaxin acts alone or in combination with other hormones in its effects on connective tissues. In the non-pregnant state, ligament growth can be induced in an oestrogen primed animal only following administration of relaxin (Wahl et al., 1997).

Progesterone inhibits collagenase activity but relaxin has no direct inhibitory effect. Kapila and Xie (1998) looking at remodelling in the fibro-cartilaginous disc of the temporo-mandibular Joint implicated relaxin alone, or again with beta oestradiol as a factor responsible for collagen remodelling. Luque et al., (1966, 1998) and Samuels (1998) both found that relaxin plays an important part in collagen remodelling and reorganisation in rat tissues. Kristiansson (1996) linked high levels of relaxin in the early part of IVF pregnancies to back pain radiating to the sacrum but failed to suggest the mechanism for the symptoms other than citing ligament laxity as being the problem.

The recent work of Hsu and his research team, has finally demonstrated specific receptor sites for relaxin in various tissues. With the identification

of these receptors it is now possible to investigate specific cells and tissues that are responsible for relaxin in diverse pathological and physiological tissue states (Hsu et al., 2003).

## **The effects of other hormones on connective tissues**

That connective tissue components, in particular collagen, are affected by various hormones has been known for a long time as shown above. The exact mechanism whereby the collagens can change has been unclear until recently because the methods of analysis and the ability to isolate specific receptor sites was yet unknown.

As late as 1974 Harvey et al., looked at the effects of steroid hormones, for example the glucocorticoids, on human fibroblasts in vivo (Harvey et al., 1974). They researched the effects of cortisol and prednisilone on cell proliferation, DNA synthesis, and collagen synthesis. They found that both substances stimulated collagen synthesis at very small concentrations (0.01 micro grams per ml). Higher concentrations of the steroids caused a marked suppression of DNA synthesis but a smaller reduction of cell proliferation over a three day culture period.

Kucharz, in two papers in 1988, also explored the hormonal control of collagen metabolism (Kucharz, 1988a; Kucharz, 1988b). He reviewed the effect of thyroid hormones and thyroid stimulating hormone on collagen.

Thyroid hormone influences collagen biosynthesis and degradation, and this effect is responsible for various observed pathophysiological phenomena including alterations in the urinary excretion of hydroxyproline and hydroxylysine, pre-tibial myxoedema, impaired wound repair and other fibrosis associated processes. Thyroid stimulating hormone (TSH) also directly affects the connective tissues, with hyperthyroidism being associated with joint laxity, and may be one of the factors associated with thyroid eye disease (Daumerie, 2002; Kucharz, 2003). Other factors include the effects of thyroxine directly on the optic muscles and the deposition of a muco-polysaccharide behind the eyeball to give the exophthalmic appearance.

In another paper Kuchartz (1998b) also looked at glucocorticoids on the metabolism of collagen. He found that they decrease the amount of messenger RNA coding procollagen chains. This decrease is caused by depressed activity of the specific enzymes of intracellular stages of collagen biosynthesis. Extra-cellular maturation of collagen is affected by steroids, but changes reported depend on rate of collagen turnover. He felt that the effect of collagen degradation is a matter of controversy because unlike Unemori, (1990), he found that there was little evidence that the hormones decrease the activity of collagenase.

The main difference functionally between these hormones and oestrogen, progesterone and relaxin is that these hormones are not secreted in a



predictable and cyclic fashion as are the hormones of the menstrual cycle. Their effects, therefore, are not going to be cyclical either, and therefore they are unlikely to be related to the incidence of injury at specific times in the cycle.

## **The significance of the sex hormones on the function of the ligaments**

The mechanical strength of the pelvic ligaments supporting the female pelvic organs has long been thought of as one of the factors involved in controlling the position and function of these organs after the menopause. The sex hormones are thought to be significant in the adequate function of these ligaments and their role in preventing uterine and vaginal collapse and prolapse.

Chen's research group looked for alpha and beta oestrogen receptors in the vaginal walls and utero-sacral ligaments in pre-menopausal and post-menopausal women (Chen et al., 1999). They analysed the expression of messenger RNA for the receptors, and concluded that alpha and beta oestrogen receptors were expressed in the vaginal walls and utero-sacral ligaments of pre-menopausal women, but that the beta receptor cells were absent from the vaginal walls after the menopause, thus predisposing these women to pelvic organ collapse.

Stewart's research group studied collagen types I and III in uterine leiomyomas in various phases of the menstrual cycle (Stewart et al., 1994). They posed the question that as different hormones are present at various phases of the cycle did one hormone have more or less an effect on type I and type III collagens than another hormone? Uterine leiomyomas contain abundant quantities of extra-cellular matrix, in particular collagen types I and III and fibronectin. From looking at the levels of messenger RNA for these proteins, they concluded that leiomyomas show increased levels of messenger RNA for collagen types I and III compared to normal myometrium. However, this difference is manifested only during the proliferative phases of the menstrual cycle. These findings suggest that leiomyomas are more sensitive to oestrogen levels which are higher in the proliferative phase of the cycle than the normal myometrium.

As indicated above, various researchers had investigated the therapeutic uses of relaxin in skin diseases, notably scleroderma (Gavino and Furst 2001). Varila et al., (1955), looked at the effect of topical oestradiol on the skin collagen in post-menopausal women and found that it increased the amount of skin collagen and stimulated Type I and Type III collagen synthesis both in the quality and quantity of fibrils.

The effects of the sex hormones on collagen have the potential for widespread effects not just confined to pelvic ligaments or to reproductive

tissues. Abubaker et al., (1966) concluded that sex steroids have an effect on the collagen and protein content of the articular discs of the temporo-mandibular joint in rats as indicated by the difference in the values between control males and females and by the disappearance of this difference on castration of both male and female animals. This was also manifested by the significant effect of oestradiol on collagen content of castrated male rats, and by the effect of oestrogen combined with progesterone on the protein content of castrated females.

It has been postulated that the capsules of the spinal apophyseal or facet joints also contain receptors for  $17\beta$  oestradiol. Nadaud et al., (2001) showed that a degenerative spondylolisthesis is four to five times more common in females than in males suggesting that hormonal influences account for the gender difference. The researchers investigated facet joint capsular ligaments for the presence of such receptors. They found that none of the specimens contained receptors and that if hormones do play a role in the development of a degenerative spondylolisthesis, then it is probably not one of the sex hormones that is involved. It therefore still remains to be seen if relaxin receptors can be identified in these sites and are therefore implicated in the development of the condition.

Brynhildsen et al., (1998) tried to implicate hormone replacement therapy, HRT, as a risk factor for low-back pain amongst menopausal women, and felt that there was a significant, albeit weak, positive association between

current use of HRT and low-back pain. They felt that the results could not be explained by differences in occupation, smoking habits, or current physical activity. Instead they speculated that the hormonal effects on joints and ligaments may be involved.

## **Summary**

The literature review examined the current state of knowledge of the physiology of the sex hormones involved in the menstrual cycle. It then reviewed the evidence demonstrating the presence of specific receptor sites in connective tissues and skeletal muscles, and went on to review the literature showing the effect these hormones have on these tissues. The literature review investigating the incidence of injury in relation to the menstrual cycles of athletes, as shown in the in the previous chapter, revealed that many thousands of female athletes injure themselves each year in sport compared to males (Toth and Cordasco, 2001; Arendt et al., 2002).

If, in fact, these women are injuring themselves because they are more vulnerable to injury, then potentially it is the change in joint laxity caused by the change in the supporting tissues at different phases of the menstrual cycle that might be contributing to these events.

## **The aim of the study**

The aim of the laxity study is to investigate the potential change in laxity as demonstrated at a peripheral joint at different phases of the menstrual cycle.

## **The Hypotheses**

The main experimental hypothesis states that women with a normal and predictable menstrual cycle experience changes in joint laxity. The null hypothesis is states that there is no relation between the phases of the cycle and joint laxity.

The second experimental hypothesis states that if these changes exist they correlate with changes in the levels of one or more of the circulating sex hormones .The null hypothesis states that there is no relationship between joint laxity and the levels of the circulating sex hormones.

## **Method and research protocol**

Sixty female volunteers were recruited from the clinic at The British School of Osteopathy, students from the British School of Osteopathy, and patients attending the author's private osteopathy practice. The criteria for inclusion in the study were that they had to have a predictable menstrual cycle and that they had not used oral contraceptives or any other hormone preparation for at least three months prior to being tested. It was also important that there was no history of inflammatory joint disease or trauma to the joint under test for the subject to be included in the study.

## **Ethical considerations**

All subjects were given an appropriate information sheet about the relevant study for them, and then they were required to sign a consent form a copy of which was given to them to keep. Ethical approval for the studies had been obtained from the Ethics Committee of The British School of Osteopathy and from the Ethics Committee of The Chelsea and Westminster Hospital where the blood samples obtained from the laxity study were to be analysed.

The female subjects were given a menstrual diary to keep for three months prior to being measured for the first time. This was despite the fact that only women who thought that they had a regular and predictable cycle were included in the study group. The rationale behind this is that many women who think that they have a regular and predictable menstrual cycle are not regular at all, but never know because they have never checked themselves accurately. It was essential for the study to measure women on specific days in the cycle; therefore, a calendar was used to ascertain the regularity and predictability of the subjects to be included in the experimental group.

Of the 60 original volunteers, 43 were eventually rejected as they were not found to have a predictable and reliable menstrual cycle after all. For the purposes of the study it was considered essential to measure the subjects on the exact day specified in their cycle. If they were found to menstruate earlier or later than expected by more than one day, then the results obtained had to be abandoned as they were not true representative readings of what was happening on a specific day. Thus reliable data were forthcoming from only 17 participants.

The average age of the experimental group was 25.35 (st dev 7.99 years; sem 2.30 years ).

Twenty male volunteers were also chosen from the student population of the British School of Osteopathy to act as a control group for the first part of the study relating to changes in laxity and phases of the menstrual

cycle. The only inclusion criteria for this group were that there should be no history of any inflammatory joint disease and no history of trauma to the joint under examination.

The average age of the male subjects forming the control group was 28.70 years (st dev 5.88 years; sem 1.43 years).

Continuous quantitative data were collected from the subjects using the Hyperextensometer as described before. The female subjects either came to the author's private practice or to the British School of Osteopathy for measurement, or they were visited at their homes and measured on the following days in the menstrual cycle.

Day 3 to ascertain a base level of hormone readings

Day 12 at the expected pre-luteal surge for oestrogen levels

Day 21 at the expected progesterone peak

Day 27 at the expected relaxin peak.

At each test session the subjects were first measured using the Hyperextensometer described above. Three readings were obtained and the averages recorded. Then 2 ml of blood was taken from the median cubital vein of the non-dominant arm.

The blood was separated and the serum was labelled and stored at minus 2° C in a refrigerator especially purchased for this purpose kept in



a store room under lock and key at the British School of Osteopathy. Eventually at the end of the study the samples were sent to the biochemistry laboratory of the Chelsea and Westminster Hospital for analysis. Thus for each phase of the cycle an understanding of the data relating to the effects of the hormone relative to the joint laxity was obtained.

The control group subjects were measured using the Hyperextensometer once per week at seven day intervals for four weeks at the British School of Osteopathy. As males are not subject to cyclic changes in their hormones in the same way that females are, and as they were only being studied to ascertain if there was a change in male joint laxity over a four week period, it was not deemed necessary for them to undergo the blood analysis.

The raw data were entered onto a Microsoft Excel spreadsheet for data analysis. SPSS version 13.0 and Stats Direct version 2.4.5. were also sometimes used in addition to the Excel data analysis package.

In all statistical analysis a value of  $p < 0.05$  was deemed significant,  $p < 0.001$  highly significant and  $p < 0.0001$  extremely significant.

## Results and statistical analysis

### Laxity and the control group

The Shapiro Wilk test showed the data obtained from the control group to be normally distributed ( $p=0.1359$   $n=20$ ).

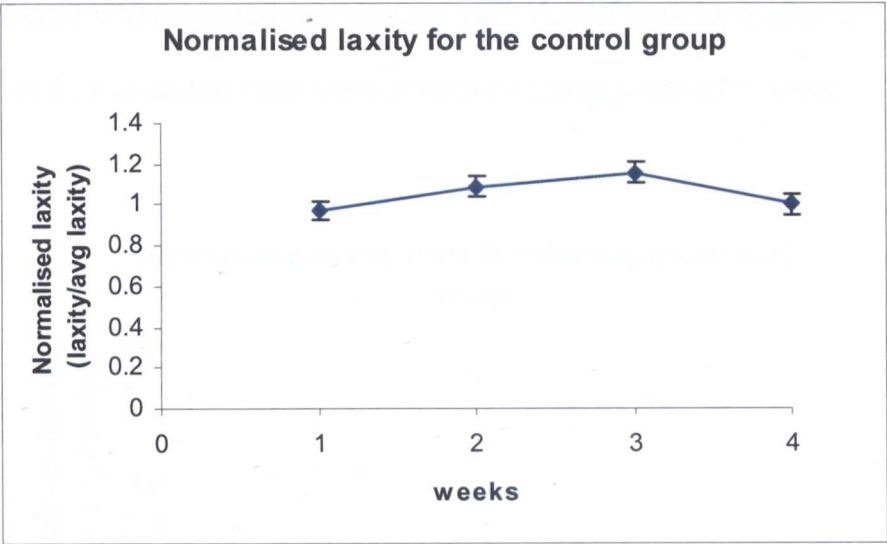


Figure 20: The changes in laxity in males over four weeks

Figure 20 shows that the male subjects did exhibit a change in laxity in the joint measured over a four week period.

The data was normalised by dividing the laxity readings at each phase by the mean of the readings to remove variation between subjects whilst leaving the variation between the phases.

A repeated measures ANOVA test applied to the data showed that these changes were not considered statistically significant ( $p=0.7394$ ). With a  $p$  value  $> 0.05$  a post hoc test was not considered necessary.

### Laxity and the experimental group

The data from the experimental group were subjected to a Shapiro-Wilk test which showed there was no evidence of non-normality ( $p=0.632$   $n=17$ ) and so the data were analysed using parametric tests.

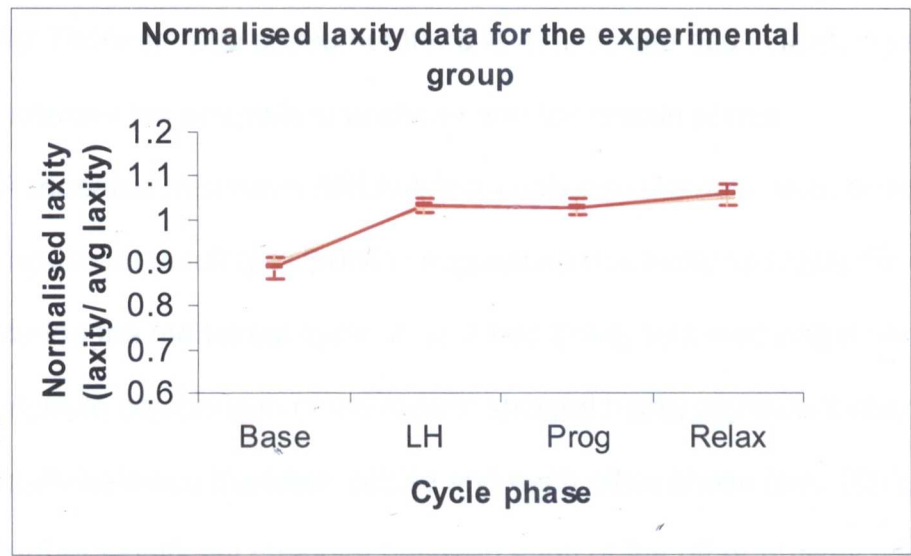


Figure 21: The change in laxity in females over the four weeks of a menstrual cycle

The data was normalised by dividing the laxity readings at each phase by the mean of the readings to remove variation between subjects whilst leaving the variation between the phases.

Figure 21 shows the normalised laxity data for the experimental group plotted against menstrual cycle phase. Base refers to the Base phase of the cycle between days 1 and 3 when the levels of the circulating sex hormones are not significant. LH refers to the phase of the cycle around day 14 when the surge of Lutenising Hormone causes ovulation to occur. Prog refers to the phase in the cycle around day 21 days, when the expected peak in the levels of circulating Progesterone occurs, and Relax refers to the time around day 27 when the levels of circulating relaxin are at their highest.

The chart shows a rise in laxity between the base phase and the LH phase. There is a hardly discernable change between the LH phase and the Progesterone phase but there is a moderate rise in laxity again between the progesterone phase and the relaxin phase.

A repeated measures ANOVA test applied to the data gave an extremely significant result ( $p < 0.0001$ ) suggesting that laxity changes did occur during the menstrual cycle. A post hoc Tukey test involving a pair wise multiple comparison of the means showed highly significant changes in laxity between the base phase and each other phase ( $p < 0.001$ ), and no further significant changes between each of the other phases when tested against each other ( $p > 0.05$ ). The results indicate that laxity as measured at the first metacarpo phalangeal joint of the experimental

subjects changed during the menstrual cycle, and thus the main null hypothesis can be rejected.

Oestrogen in levels in the experimental group:

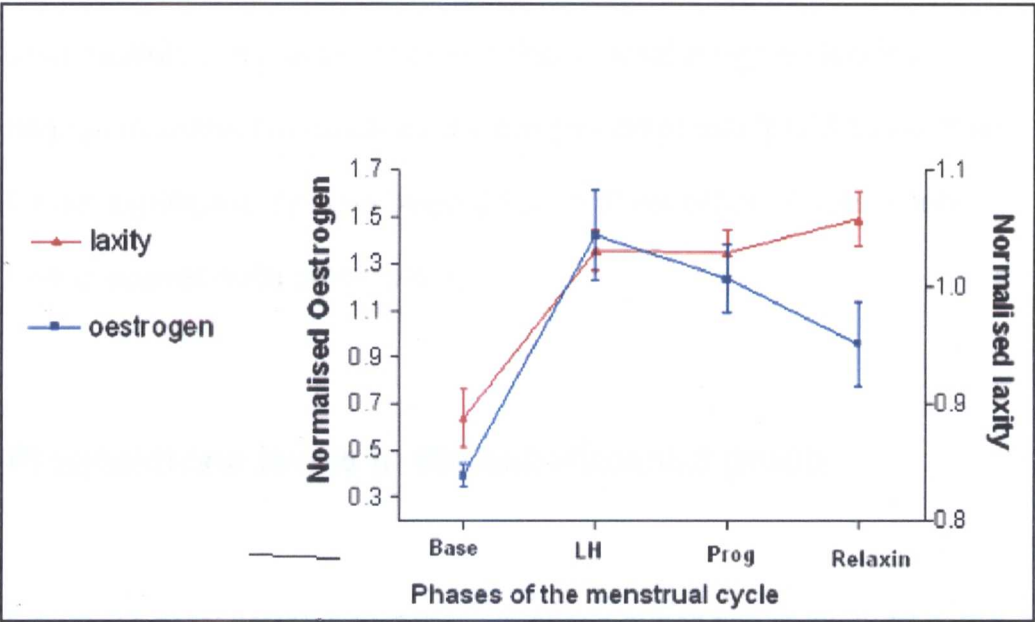


Figure 22: The comparative plot of change in laxity and oestrogen across one menstrual cycle.

Figure 22 demonstrates that laxity rises as plasma oestrogen rises in the first phase of the cycle. This confirms the expected rise of plasma oestrogen in the follicular phase of the cycle when the developing follicle is producing the pre luteal surge of oestrogen needed for ovulation. Without the rise in oestrogen there can be no corresponding rise in Lutenising hormone, LH. After this first phase of the menstrual cycle, oestrogen levels fall as the luteal phase of the cycle proceeds and the production of ovarian oestrogen is withdrawn. The scatter plot further demonstrates, however, that laxity does continue to rise in the next phases independent of the oestrogen fall.

A repeated measures ANOVA test applied to the data showed an extremely significant difference in oestrogen levels between the various phases of the cycle ( $p=0.0001$ ). A post hoc Tukey test involving a pair wise multiple comparison of the means showed a highly significant change in oestrogen levels for the base to LH phase ( $p<0.001$ ) with no further significant changes between each of the other phases when tested against each other ( $p>0.05$ ).

### **Progesterone levels in the experimental group**

Figure 23 demonstrates that there is only a minor rise in laxity from the LH to the Progesterone phase of the cycle despite the rapid rise in levels of progesterone in this phase.

A repeated measures ANOVA test applied to the data demonstrated that there was a extremely significant ( $p<0.0001$ ) change in progesterone levels across the cycle.

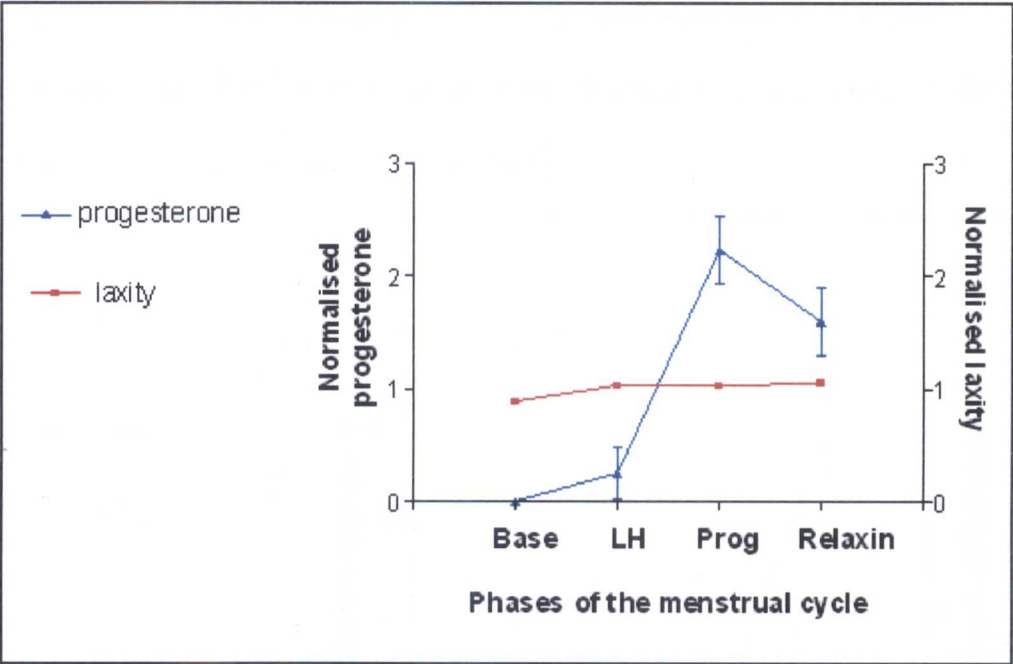


Figure 23: The comparative plot of change in laxity and levels of progesterone across one menstrual cycle

A post hoc Tukey test involving a pair-wise multiple comparison of the means demonstrated extremely significant differences ( $p < 0.0001$ ) in progesterone levels between the base versus progesterone phases the LH versus progesterone phases ( $p < 0.0001$ ), and base versus relaxin phases ( $p < 0.0001$ ). Comparisons between each of the other phases was not significant ( $p > 0.05$ ).

### Relaxin levels in the experimental group

Figure 24 demonstrates that laxity undergoes a very small change in the last phase of the cycle and that the levels of relaxin only really appear to



become apparent in this phase of the cycle .A repeated measures ANOVA applied to the data showed that there was a extremely significant change in laxity at this time ( $p<0.0001$ ).

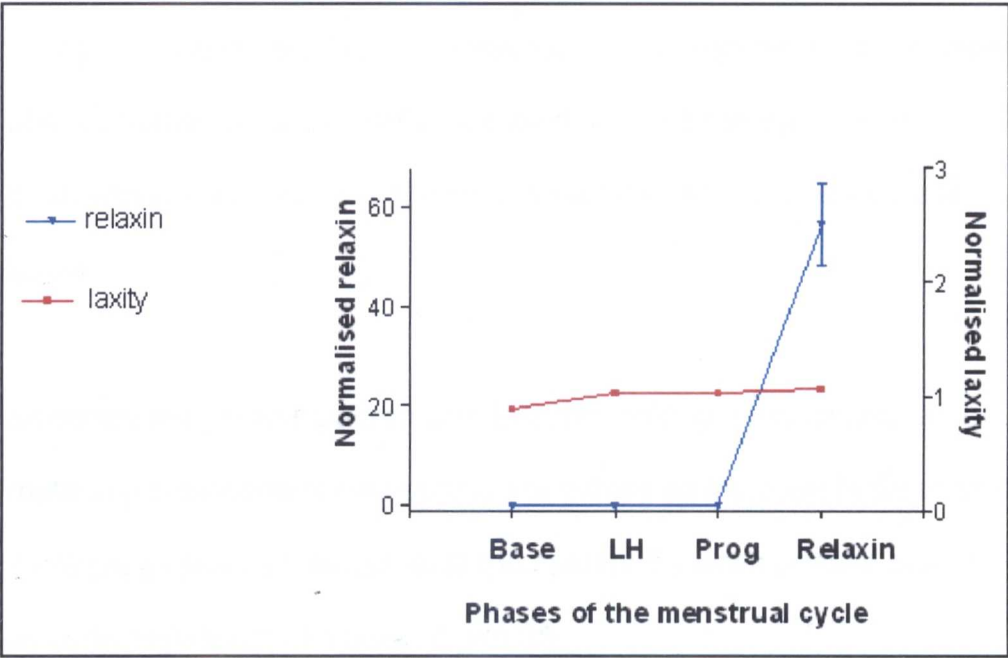


Figure 24: The comparative plot of change in laxity and relaxin across one menstrual cycle

A post hoc Tukey test involving a pair wise multiple comparison of the means highlighted an extremely significant change in relaxin levels when comparing the base and relaxin phases ( $p<0.0001$ ); the LH and relaxin phases ( $p<0.0001$ ); and the progesterone and relaxin phases ( $p<0.0001$ ). Comparisons between all other phases were not significant ( $p>0.05$ ). Thus it can be seen that the laxity varied as the levels of specific circulating sex hormones varied and thus the second null hypothesis can be likewise rejected.

## Discussion and Conclusions

A number of conclusions can be drawn from the above.

Firstly not only did the laxity of every subject change between the various phases of the menstrual cycle, but the degree of change differed depending on the phase of the cycle when the measurements were taken.

Secondly the general pattern was that the mobility as measured at the metacarpo-phalangeal joint seemed to exhibit an increase in laxity as the cycle progressed. However, only the change between phases one and two was significant at a level of  $p < 0.05$ .

Thirdly the presence of a specific hormone in a specific phase was probably the important contributing factor relating to the change in laxity within the phase. This related particularly to the oestrogen findings illustrated in figure 22.

The results from this study suggest that the laxity does change during each phase in relation to the presence of the hormones concerned, and the most significant change was found between the base and LH phases of the cycle when the level of circulating oestrogen was rising. It strongly suggests that oestrogen is an important hormone responsible for the laxity effect found. Progesterone was at its highest level in the third phase of the cycle but the laxity did not change significantly, suggesting that any

effects due to progesterone are minor. Surprisingly the effect of relaxin was not considered significant ( $p < 0.05$ ), because there was no significant change in the laxity found in the fourth phase of the cycle when relaxin rises to its peak. This is in contradiction to the evidence found in the literature review linking relaxin to ligamentous laxity during pregnancy (Abramso et al., 1934; Calguneri et al., 1982; Svenson et al., 1990). This might be explained by the relatively small concentrations of relaxin found in the normal menstrual cycle when compared with the greater levels found during pregnancy, especially the latter half of a normal pregnancy (Johnson et al., 1993 and 1994).

The above results correlate well with the evidence in the literature of receptors to the sex hormones in tissues that may be responsible for controlling laxity. Receptors for oestrogen are plentiful in muscles and fibroblasts whilst receptors for the other hormones are not found in such abundance (Unemori et al., 1990; Smith et al., 1990; Chen et al., 1999; Slauterbeck et al., 2002).

The relation between the hormones and a propensity to injury may well relate to the effects of these hormones on skeletal muscles and on collagenous connective tissues, and the extent to which those tissues contribute towards the protection mechanisms preventing the subject from overstrain and damage situations.

The literature review demonstrated that the effects of oestrogen hormones on skeletal muscles are profound, and the greater the number of receptors the greater is the possibility of the direct action of the hormone on the muscles concerned, especially if they are slow twitch fibres as are often found in postural muscles. This may be important in relation to the acute pain incidence reported in the pilot study when the postural muscles are called into play as part of the overall protection mechanism. It is the effects of the receptors to the various hormones which can contribute towards the strength or weakness of individual muscles, and therefore to the degree of protection available at various phases of the menstrual cycle.

The fluctuations in female steroid hormones affect the autonomic nervous system and metabolic functions as well as muscle function (Florini, 1987). Whilst the skeletal muscles are under the control of the central nervous system, the blood vasculature to the skeletal muscles is under the control of the autonomic nervous system and so again there is a positive link between the level of the female steroid hormones and way skeletal muscles function.

The literature review showed that the evidence relating to skeletal muscle strength is not conclusive. Onambele (2002) cited sources with negative correlation between skeletal muscle force and menstrual cycle phase, no correlation and positive correlation between the cycle phases. She

maintained that it is unlikely that the reasons for these variations is because of the variations in methods of muscle strength measurement and cycle phase, and variations in oestrogen levels in different subjects in the same phase of the menstrual cycle. More needs to be done in this field to obtain a clearer picture about the relationship between oestrogen, muscle strength, and joint laxity in the menstrual cycle.

The evidence linking the female steroid hormones and the collagenous connective tissues revealed an abundance of receptors for oestrogen and relaxin in various sites both genital and extra genital. Current thought is that relaxin acts through an interplay between the synthesis and demolition of collagen (Unemori et al., 1990) and that its effects might be synergistic with the ovarian steroids. Relaxin receptors have been found in the fibro- cartilaginous discs in the temporomandibular joint (Kapila and Xie, 1998), the brain, renal and cardiovascular tissues (Hsu et al., 2002), and in spinal apophyseal joints (Nadaud et al., 2001).

The widespread presence of these receptors together with the almost ubiquitous presence of oestrogen receptors does suggest that these hormones can have a global effect of many tissues throughout the body, not the least of which are the skeletal muscles and connective tissues.

The findings in this chapter have revealed a definite link between the physiological levels of various reproductive hormones in the female and

laxity as measured at the first carpo-metacarpal joint in normally menstruating subjects.

# ***Chapter Four:***

# ***The Pregnancy Study***

## **Introduction**

Pregnancy has traditionally been accepted as a time when the joints, especially the pelvic joints, become more lax (Abramson et al., 1934; Calgneri et al., 1982; Johnson et al., 1994). Obstetric text books refer to changes in ligamentous laxity without reference to the studies quantifying these changes (Llewelyn Jones, 1997; Bennet, 1993). The pregnancy study examined a varied population of primaraparous and multiparous women to see if this laxity can be quantified and to study women during the different weeks of pregnancy to ascertain if the change in laxity was uniform throughout the pregnancy or if in fact they were more likely to undergo changes in laxity in one particular part of the pregnancy compared to any.

## **The literature review of the hormone changes of pregnancy**

This series of experiments investigates the changes in laxity that take place specifically during pregnancy and during the post-partum period.

The following paragraphs therefore review the roles of the sex hormones on connective tissues at this time.



## The role of progesterone during pregnancy

Progesterone is secreted by the corpus luteum after ovulation and it continues the preparation of the endometrium for a possible pregnancy and inhibits the development of a new follicle (Kimball,2004). If fertilisation takes place and once the placenta is established, it begins to secrete progesterone itself which supplements and eventually replaces that of the corpus luteum. The corpus luteum normally breaks down to become the corpus albicans, but if fertilisation should occur the human chorionic gonadotrophin ( HCG) secreted by the early products of conception maintains the corpus luteum until the placenta is sufficiently well developed to take over the role of progesterone production. By the 5th month of pregnancy the placenta alone is responsible for progesterone secretion. The placenta continues to produce both progesterone and oestrogen for the duration of the pregnancy and the levels continue to rise until just before the birth.

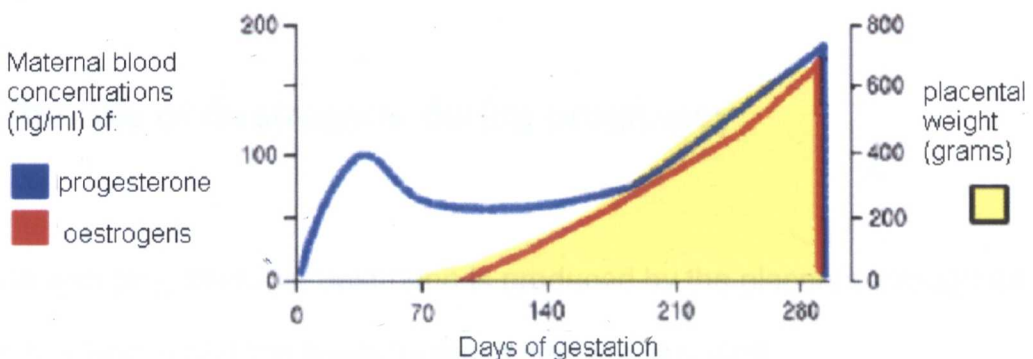


Figure 25: The rising levels of Oestrogen and Progesterone during pregnancy

from Pathophysiology of the reproductive system .Colorado State University Web Site  
By Bowen RA <http://arbl.cvmbs.colostate.edu/hbooks/pathphys/reprod/index.html>

Figure 25 shows the average growth in the level of progesterone within the body during a pregnancy. A minimum level of about 10 ng.ml<sup>-1</sup> is required to sustain a pregnancy through the very early stages (Bennet, 1993). At the end of the pregnancy, the levels of progesterone secreted by the placenta will decrease. It is this action that stimulates the beginning of the contractions that lead to birth.

Progesterone has a number of important physiological functions during the pregnancy. Amongst the functions are the maintenance and development of the endometrial lining in conjunction with oestrogen; maintaining the function of the placenta and stopping the uterus undergoing spontaneous contractions; stimulating the growth of breast tissue and preventing lactation until after the birth, again in conjunction with oestrogen; strengthening the cervical mucus plug helping to maintain the pregnancy for the full 40 weeks, and strengthening the muscles of the pelvic floor in preparation for labour ( Khan-Dawood et al., 1989).

### **The role of Oestrogens during pregnancy**

As with progesterone, oestrogen is produced by the placenta throughout a pregnancy and the levels increase steadily until birth.

Each hormone has a specific role to play during pregnancy however there can be a considerable overlap of functions and often the hormones oestrogen and progesterone act in concert (Kimball,2004).

Oestrogen plays a very important role in the development of the fetus; without oestrogen, the lungs, kidneys, liver, adrenal glands and other organs would never be triggered into maturation. In fact, the placenta itself would never grow and operate properly if not for oestrogen (Tortura and Grabowski, 2000).

Oestrogen also helps to trigger the maturation of reproductive organs, and help in the development of sexual characteristics. It assists in the lactation process and regulates bone density in a fetus. Along with progesterone it maintains the endometrium during pregnancy and is responsible for the promotion of blood flow within the uterine walls. Lastly it protects female foetuses from the effects of maternal androgens (Tortura and Grabowski, 2000).

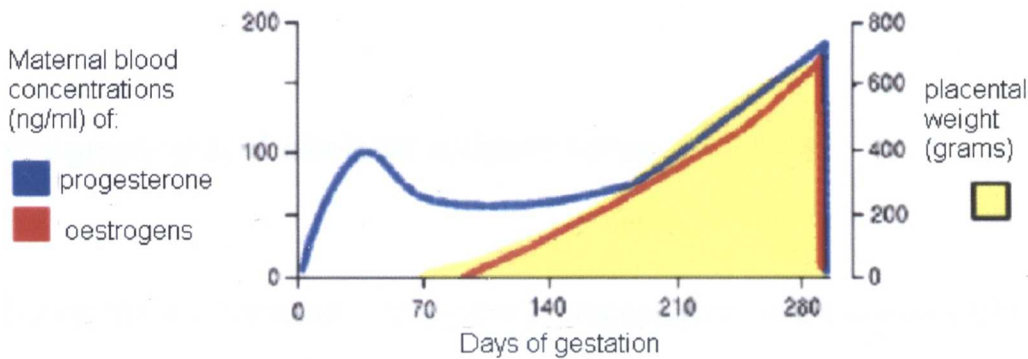


Figure 25: The rising levels of Oestrogen and Progesterone during pregnancy

Pathophysiology of the reproductive system .Colorado State University Web Site  
By Bowen RA <http://arbl.cvmbs.colostate.edu/hbooks/pathphys/reprod/index.html>

## **The role of Relaxin during pregnancy**

Relaxin has been traditionally regarded as the main hormone affecting connective tissue and thus causing joint laxity during a pregnancy (Weiss, 1991, Weiss, 1993, Weiss et al., 1995). The expression of relaxin receptors is controlled by oestrogen (Tan et al., 1999, Siebel et al., 2003). Oestrogen priming increases the release of endogenous relaxin, increases the response of the target organs to relaxin, and increases the number of relaxin binding sites on human smooth muscle and myometrium (Bryant-Greenwood and Schwabe, 1994). It is not surprising then that as the levels of oestrogen rise, so do the levels of relaxin. Likewise after the delivery, oestrogen levels fall rapidly with the absence of the placenta, and serum relaxin levels disappear within 48 hours post-partum (Eddie et al., 1989).

## **Pregnancy and skeletal muscle force**

During the first trimester of pregnancy, progesterone levels are very high so as to enable the pregnancy to continue. They continue to rise throughout the pregnancy. From the end of the third trimester onwards a continuous rise in maternal oestriol levels is seen but there is little evidence to support correlating change in muscle performance and strength (Elliott, 2001). This research seems to show that there is no

distinct link between muscle strength and hormone levels during pregnancy.

Therefore it is likely as seen from the above that the main hormone that affects and controls connective tissue tone and the control of mobility of the joints during pregnancy is firstly oestrogen because it is necessary to prime the tissues and aid the expression of the relaxin receptors, and relaxin itself because there are specific receptors for this hormone in connective tissues within the pelvis and elsewhere. There is little evidence found in the literary review that progesterone has anything to do with controlling either skeletal muscle tone or the tone of the ligaments.

## **The Hypotheses**

The experimental hypothesis states that women undergo changes in joint laxity as they proceed through the 40 weeks of pregnancy. The null hypothesis states that there is no change in joint laxity during the 40 weeks of pregnancy.

The second experimental hypothesis states that if this change is present it varies according to the number of weeks of her pregnancy. The secondary null hypothesis states that the rate of change is uniform throughout the pregnancy.

The third experimental hypothesis states that a woman's pre-pregnancy laxity has an influence on her rate of change, and that if she is hypermobile at the start of the pregnancy her rate of change is different to that of a subject who is normal or hypomobile compared to the others in the group under test. The third null hypothesis states that there is no difference between the two with regard to rates of change.

## **Method and research protocol**

Forty subjects were recruited from the author's private osteopathic practice. The average gestation at the start of the study ranged from 6 weeks to 30 weeks with a mean of 15.69 weeks. The subjects were measured up to 39 weeks of gestation. The minimum number of readings per subject was 3 and the maximum was 8 with an average of 5 readings overall. Of the group under study 23 were primiparous pregnancies and 17 were multiparous pregnancies.

The only exclusion criteria were that there should be no history of repeated spontaneous abortions, and that they should have no history of bone or joint pathology prior to the study. A history of repeated spontaneous abortion can have many causes one of which may be unusual or insufficient hormones to maintain the pregnancy. Therefore these subjects were excluded so as to ensure only those subjects with as near normal hormones were included. Likewise subjects with joint

pathology may exhibit unusual and non-representative changes in their joints and connective tissues and give abnormal results.

The volunteers were given an information leaflet explaining the purpose of the study. They then signed a consent form a copy of which was given to them to keep. Ethical approval for the study had already been obtained from the ethics committee of The British School of Osteopathy.

The first metacarpo-phalangeal (MCP) joint of the subject's dominant hand was measured every four weeks throughout the pregnancy using the Hyperextensometer as before. Three readings were taken each time and the readings were averaged and recorded as mentioned before.

## **Results and statistical analysis**

The data were subjected to a Shapiro-Wilk test to ascertain if the data obtained were normally distributed. The results showed that the data were unlikely to be from a normal distribution ( $p=0.0016$ ,  $n=185$ ) therefore non-parametric tests were used for statistical analysis.

In order to investigate the first hypothesis, two scatter plots Figures 26 and 27 were produced from the raw data, one including and one excluding a subject who was considerably more mobile than the rest of the experimental group at the start of the experiment. This was undertaken to give a more representative view of the laxity obtained in a normal pregnancy.

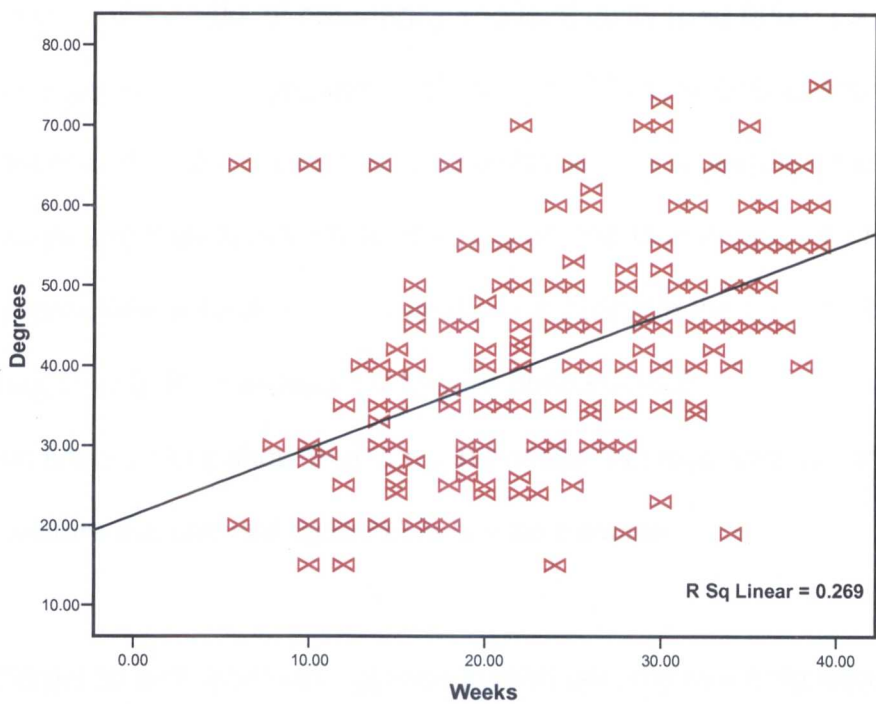


Fig 26: Scatter plot weeks of gestation versus degrees of laxity including the hypermobile subject

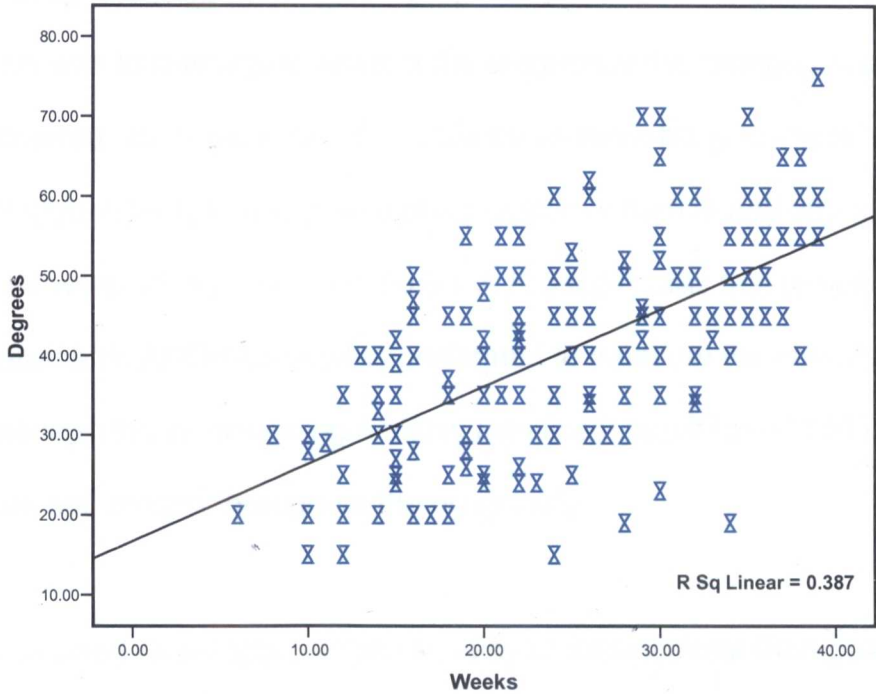


Fig 27: Scatter plot weeks of gestation versus degrees of laxity excluding the hypermobile subject



The correlation calculation in both cases showed a strong correlation between the weeks of pregnancy and joint laxity ( $p < 0.0001$ ;  $r = 0.518$  with the hypermobile subject included, and  $p < 0.0001$ ;  $r = 0.622$ , with the hypermobile subject excluded respectively). The strength of the positive relationship between laxity and weeks of gestation improved when the hypermobile subject was excluded ( $r^2 = 0.269$  with the hypermobile subject,  $r^2 = 0.387$  without the hypermobile subject).

The scatter plots show that laxity increases as pregnancy progresses therefore the first null hypothesis can be rejected.

In order to investigate the second hypothesis, the raw data were separated into four groups being 0-10 weeks of pregnancy, 11-20 weeks of pregnancy, 21-30 weeks of pregnancy, and 31-40 weeks of pregnancy. This was to investigate when in the pregnancy the changes in laxity occurred and to ascertain if a subject was more likely to experience a change in laxity in one phase of a pregnancy than at any other. As the data were not normally distributed, A Kruskal-Wallis test (a non-parametric ANOVA test) was performed to measure the variance between the weeks giving a highly significant result ( $p < 0.0001$ ). Laxity was not uniform throughout the pregnancy.

A squared ranks approximate equality of variance test (non-parametric equivalent of the Tukey post hoc test) performed on the data to ascertain exactly where the variance between the weeks was occurring produced a

highly significant result ( $\text{Chi}^2 = 9.631$ ,  $\text{df} = 3$ ,  $P = 0.022$ ). This test showed highly significant changes in laxity between weeks 11-20 and 20 -30 ( $p=0.045$ ) and between weeks 20 -30 and 30 -40 ( $p=0.003$ ). However, none of the other comparisons was found to be significant. Therefore the second null hypothesis can be rejected as the change was not uniform throughout pregnancy.

In order to investigate the third hypothesis the data were subjected to a regression analysis to determine if the rate of change remains uniform throughout the pregnancy, and also to see if those subjects who were apparently hypermobile at the start of the study compared to the rest of the group changed at a different rate

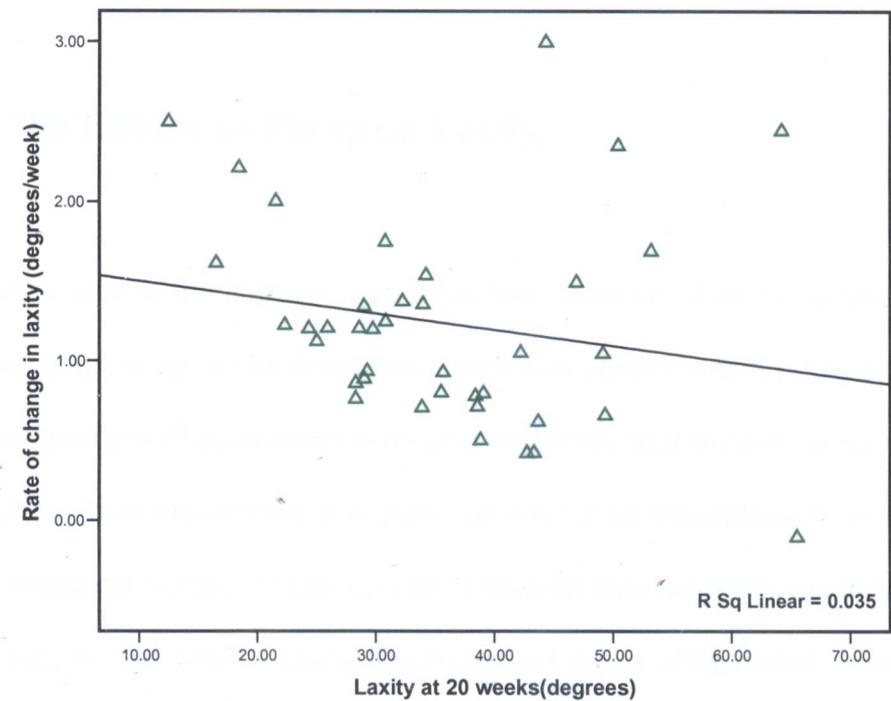


Figure28: The relationship between laxity at week 20 and the rate of change during the pregnancy expressed as degrees per week.

Figure 28 shows the relationship between laxity at 20 weeks, the mid point in a 40-week gestation, and the rate of overall change as expressed as degrees per week.

The scatter plot shows that in some subjects if the subject is very lax at 20 weeks her rate of change was small, conversely if her rate of change was high then she was relatively hypomobile at 20 weeks ( $p=0.2503$ ,  $r^2=0.035$ ). However this was not the case with every subject as some subjects who were very lax at the start of the experiment also had a high rate of change. This is confirmed by correlation analysis which revealed the correlation coefficient was not significantly different from zero ( $r=0.186$ ) and that there was no significant relationship between rate of change in laxity over the pregnancy and laxity at week 20. Therefore the experimental hypothesis for the third hypothesis can be rejected.

## **The Effects of Parity on Laxity**

A review of the literature revealed little evidence that parity affects laxity in pregnancy. It has been the author's experience that patients are regularly told by obstetricians and midwives that once they have had one baby then the second pregnancy is easier as the tissues have been stretched before. There seems to be with little scientific evidence to support the claims. Using the National Library of Medicine data base and Medline only two papers were found that mentioned parity specifically in association with joint laxity in pregnancy. Calguneri et al., (1982),

compared primiparous and multiparous patients using a finger hyperextensometer similar to that used above. A significant difference in laxity in women having their second baby compared to those having their first child was reported. The authors went on to state that further increase in laxity occurred in subsequent pregnancies, but data were not shown and the authors did not give a detailed physiological explanation of the changes. Another paper in 1996 (Schauberger et al., 1996) looked at laxity in peripheral joints but found no correlation with serum relaxin levels or with parity again the cause of any changes during pregnancy was not known. These were the only two papers found that even mentioned laxity and parity and the results disagree.

The data obtained from the pregnancy study were analysed to see if there was a difference between the laxity of the primiparous and the multiparous subjects.

Of the 40 subjects studied, 23 were primiparous women and 17 were multiparous women. Of the multiparous subjects 2 were pregnant for the third time. All of the subjects had conceived by natural methods (as opposed to HRT pregnancies) and all were singleton pregnancies.

## The Hypotheses

The experimental hypothesis states that there is a significant difference in laxity between primiparous and multiparous subjects under test. The null hypothesis states that there is no difference in laxity between the two groups.

The second experimental hypothesis states that if this change is present it varies according to the number of weeks of her pregnancy. The secondary null hypothesis states that the rate of change is uniform throughout the pregnancy.

In order to examine the first experimental hypothesis, a Mann Whitney U test was used to compare the means of the laxity readings obtained from the two groups. It was decided once again to exclude the obviously hypermobile subject from the analysis as it was thought that her obvious laxity may have been a confounding variable. Using the observations made across the pregnancies from both primiparous ( $n_1$ ) and multiparous ( $n_2$ ) subjects, ( $U = 2853.5$ ,  $n_1 = 105$ ,  $n_2 = 71$ ) the results were found to be significant ( $p = 0.0081$ ) for a two-tailed test.

The mean laxity was significantly different in each group.

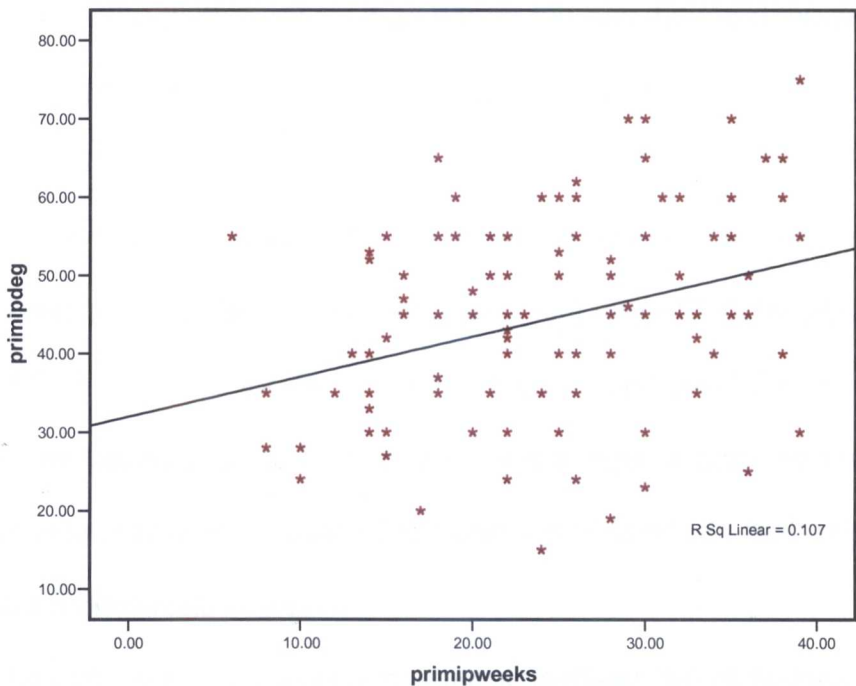


Figure 29: A scatter plot to show weeks of gestation versus degrees of change for the primiparous subjects minus the hypermobile subject

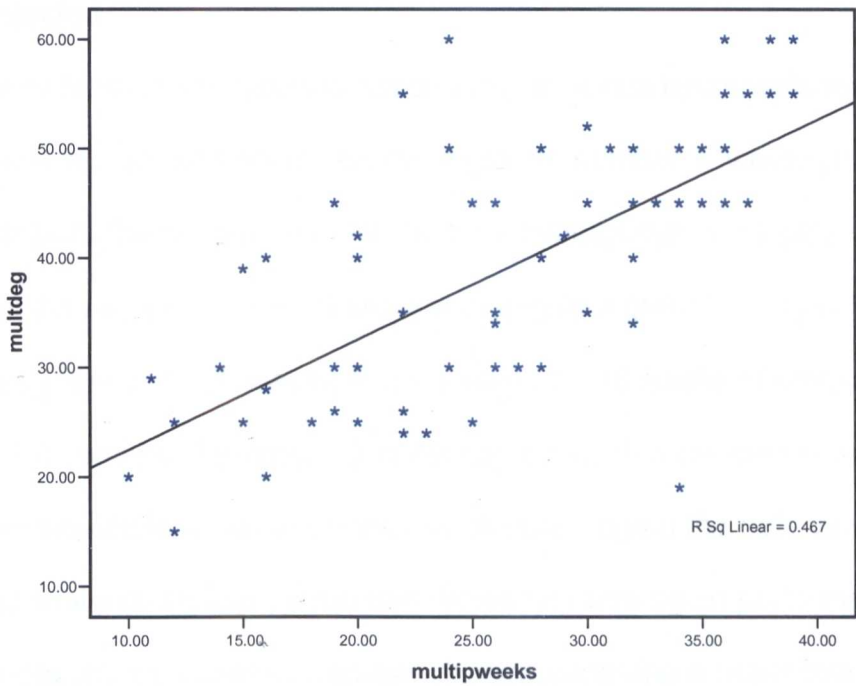


Figure 30: A scatter plot to show weeks of gestation versus degrees of change for the multiparous subjects.

Two scatter plots were produced from the raw data and these are represented in figures 29 and 30 respectively.

The correlation calculation in both cases suggested a strong correlation between the weeks of pregnancy and joint laxity for both groups ( $p < 0.0001$ ;  $r = 0.327$  for the primiparous group and  $p < 0.0001$ ;  $r = 0.683$  for the multiparous group). There was thus a highly significant correlation between laxity and weeks of gestation ( $p < 0.0001$ ) for both primiparous and multiparous subjects.

The statistical tests and the scatter plots show that laxity increases in both groups as pregnancy progresses and thus the null hypothesis is rejected.

In order to investigate the second experimental hypothesis that laxity change if present varies according to the number of weeks, the raw data for both the primiparous (without the hypermobile subject) and the multiparous groups were separated into four groups being 0-10 weeks of pregnancy, 11-20 weeks of pregnancy, 21-30 weeks of pregnancy, and 31-40 weeks of pregnancy in the same way that the data were examined for the whole group in pregnancy. As once again the data were not normally distributed, a Kruskal-Wallis test was again performed to measure the variance between the groups giving a result that was found to be highly significant for both the primiparous and multiparous subjects ( $p < 0.0001$  for both groups). Laxity change was not found to be uniform in either the primiparous or multiparous groups.

A squared ranks approximate equality of variance test (non-parametric equivalent of the Tukey post hoc test ) was calculated for both the primiparous and multiparous subjects to ascertain where the differences appeared.

For the primiparous subjects it showed highly significant changes in laxity between weeks 1 -10 and 11 20 ( $p=0.0118$ ) and between weeks 1-10 and 31 -40 ( $p=0.0077$ ). All other comparisons were not found to be significant.

For the multiparous subjects it showed a highly significant change between weeks 1-10 and 31-40 and between weeks 11-20 and 31-40. All other comparisons were not found to be significant.

Therefore the second null hypothesis can be rejected as the change was not uniform throughout pregnancy for either primiparous or multiparous subjects.

In order to investigate the third experimental hypothesis that a woman's pre-pregnancy laxity has an influence on her rate of change, for primiparous and multiparous women, regression analysis was used to observe the rates of change in the separate groups in the same way as it was done for the analysis of the subjects in the pregnancy study looking at all of the women in one combined group.



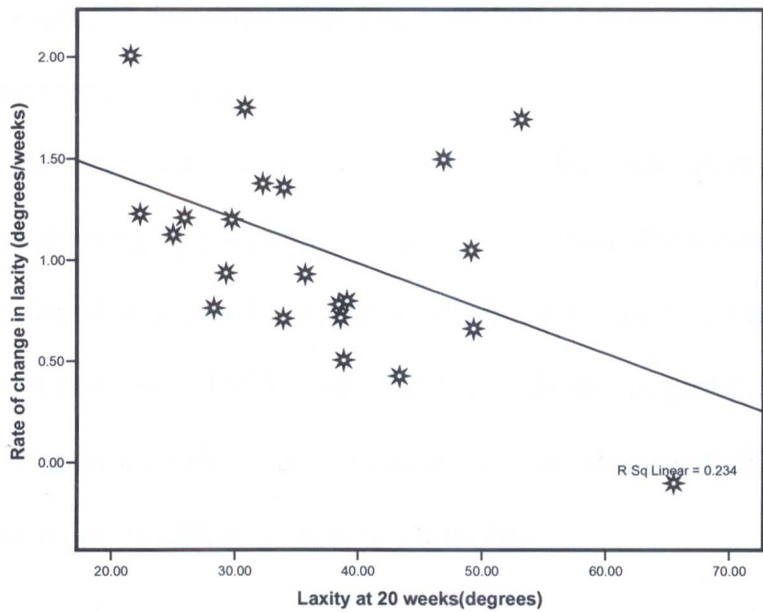


Figure 31: The relationship between laxity at week 20 and the rate of change during the pregnancy expressed as degrees per week for the primiparous subjects only.

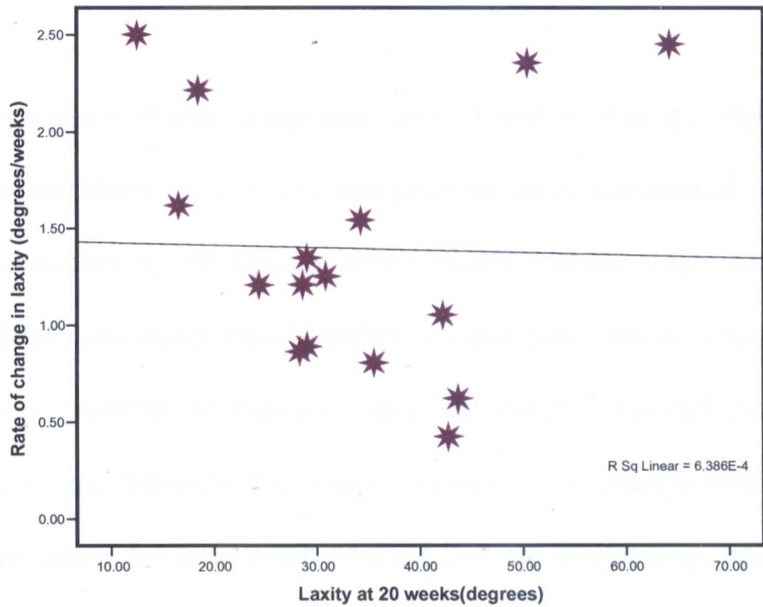


Fig 32: The relationship between laxity at week 20 and the rate of change during the pregnancy expressed as degrees per week for the multiparous subjects only.

The scatter plots shown above reveal that the rate of change between the two groups was quite different. The slope of the multiparous group is

almost flat demonstrating little correlation between laxity at 20 weeks and the rate of change.

This was confirmed with a correlation calculation that showed a strong relationship in the primiparous group (without the hypermobile subject) between the rate of laxity change and the laxity at 20 weeks ( $p=0.0194$ ,  $r=0.484$ ,  $r^2 = 0.234$ ), whilst in the multiparous group the relationship between the rate of laxity change and the change at 20 weeks was very weak, ( $p=0.926$ ,  $r= 0.024$ ,  $r^2= 0.0006$ ).

## **Discussion and conclusions**

The basis of this study was the fact that for the last eighty years researchers have noted that pregnancy is associated with ligamentous laxity and an increase in joint mobility (Hisaw, 1926; Abramson et al., 1934). However little appears to have been done to quantify this change or to understand the physiology behind the change. Do all women change? When in the pregnancy does the change occur? Is the change the same for all women or does her pre-pregnancy state have an effect on the rate of change or the quantity of the change? What effects if any does parity have on that change? These are the questions that the study tried to address.

The study addressed the first question by looking at the scatter plots obtained from the data both with and without a subject that was obviously

hypermobile compared to the rest of the group. She is identified as the outlier on the first scatter plot measured at more than sixty degrees on the y axis of the graph. It was thought that the second scatter plot would give a more representative view of a normal pregnancy. All statistical analysis was done with the hypermobile subject excluded.

The study showed that changes in laxity were not uniform throughout the pregnancy. On comparison of the mean laxity between different groups, each relating to a specific ten weeks of pregnancy, the majority of subjects changed during the middle part of the pregnancy which is earlier than the findings in the literature review (Calguneri et al., 1982; Marnach et al., 1983). Specifically the changes were highly significant between weeks 11-20 and 20-30, and between weeks 20-30 and 30-40.

The significance of the timing of the laxity change may well have an effect on the appearance of low-back pain and sacro-iliac joint pain during a pregnancy.

These problems are described as minor problems of pregnancy in standard obstetric text books (Llewelyn Jones, 1997; Bennet, 1993). The literature shows that it may well be a minor problem associated with a low rate of morbidity, and little obstetric risk, but it is a very common problem nevertheless. Research groups from around the world including Scandinavia, the UK and Australia have studied the problem. Orvieto et al., (1994), reported an incidence of 54.8 % in 449 patients; Stureson in 1997 reported an incidence of 51% in 335 patients and Mogren and

Pohjanen in 2005 reported an incidence of 72% in 891 patients (Orvietto et al., 1994; Stureson et al., 1997; Mogren et al., 2005).

The relationship between the rising levels of the hormones relaxin and oestrogen and joint laxity is potentially an important as a factor contributing towards back pain in pregnancy. The fact that these women are increasing their body weight by up to two stones (12 kg) at the end of a physiological pregnancy (Bennet RV 1993), means that as the weight increases so does the physical stress placed upon the supporting structures. Her posture will also change during the pregnancy so as to adapt to the increasing weight and this too has been attributed as one of the factors causing back pain (Dumas et al., 1995; Sihvonen et al., 1998; Gilleard et al., 2002). Lumbar apophyseal joints are not usually weight bearing joints but with the developing lordosis of the progressing pregnancy they can become weight bearing, and if the rising levels of the hormones cause a temporary laxity in the joints the instability will cause a stretch on the capsules of the joints, rich in nociceptive pain fibres, and thus low-back pain (Nadaud et al., 2001).

The development of a spondylolisthesis during pregnancy has been identified as part of pregnancy back pain syndrome for many years (Lansac et al., 1969). Pregnancy is a risk factor for those with an existing problem as well as those with the problem as yet unidentified. A risk analysis published in 1986 found that pregnancy did not constitute a major risk for the progression of an existing spondylolisthesis but it was felt to be a contributing factor to early degeneration Saraste, (1986). A

later paper in 1996 examined the evidence from 949 women and 120 men and they concluded that women who had borne children had a significantly higher incidence of degenerative spondylolisthesis than nulliparous women, (28% versus 16.7%) concluding therefore that pregnancy was an important factor in the development of a degenerative spondylolisthesis (Sanderson et al., 1996). The importance of these works is the contribution they make to an understanding of the aetiology of the problem. There are a number of reasons why there is a forward slip of one vertebra on another. The origin can be a congenital defect of the pars interarticularis or it may be a fracture of the pars. If there is excessive wear of the L4/5 disc accompanied by either a change in facet joint angles or degeneration of those facet joints at that level, this too can lead to a forward slip of one vertebra on another. In all cases the stability of the segment is controlled by the integrity of the ligaments of the spine holding bones together. If the ligaments are weakened, or if there is an increase in body weight then logically the risk of segment failure must be increased.

The pregnancy study showed that all of the women measured underwent an increase in joint laxity and that they changed in the middle of the pregnancy. An area of future research might be to devise a questionnaire to see if this also corresponded with the onset of back pain in pregnancy. In an as yet unpublished cohort analysis of patients attending the Expectant Mothers Clinic at The British School of Osteopathy, back pain was a common symptom for women presenting for treatment. The data

analysis of that study reveals that the back pain came on as early as 12 weeks in some subjects, but it took a further 10 weeks for them to seek help. This poses a question as to the relationship between the onset of the pain, the time it takes it to report the pain and her pregnancy laxity. She might be exhibiting early laxity change and therefore, she may start to exhibit back pain earlier than someone whose rate of change and therefore ligamentous laxity is slower.

The data relating to rate of change were found to be interesting. It might be assumed that if one subject was demonstrably more lax (but not hypermobile) compared to the rest of the group then she should change quickly under the influence of the ovarian hormones. Whilst some subjects did change in this expected way it was by no means universal as can be seen on the scatter plots. This is confirmed by correlation analysis which revealed the correlation coefficient was not significantly different from zero ( $r = 0.186$ ). Why this should have been apparent was not able to be explained. The clinical relevance relating to this change shows that because there was no significant rate of change difference based on the laxity at the start of the pregnancy (and presumable therefore her pre-pregnancy laxity), women who are relatively more lax at the start of the pregnancy should not necessarily exhibit any more or less tendency to back pain when compared to any other women assuming laxity and back pain are related. Future studies might explore this by trying to measure women before they conceive and then try to follow them throughout their pregnancy and measure the rates of change. An obvious place to recruit

subjects for this prospective study would be a hypermobility clinic run at a hospital rheumatology department

In the pregnancy study the parity of the subjects was considered worthy of analysis in relation to joint laxity. The literature review only produced two papers that even mentioned parity and joint laxity or back pain of pregnancy and those authors held different views (Calguneri et al., 1982; Schauburger et al., 1996).

A Mann Whitney U test showed a significant difference in mean laxity readings obtained from the primaparous and multiparous subjects ( $p=0.001$  for both). The correlation calculation in both cases suggested a strong correlation between the weeks of pregnancy and joint laxity for both groups ( $p < 0.0001$ ).

A squared ranks approximate equality of variance test showed the primaparous group exhibited change earlier than the multiparous group with change being significant between weeks 1-10 and 11-20. The multiparous group demonstrated this change between weeks 11-20 and 31-40. Both weeks demonstrated significant change between weeks 31-40.

The rate of change was different between the two groups too. The correlation calculation showed a very weak association between the rate of laxity change and the laxity at twenty weeks in the multiparous group ( $r=0.484$  for the primaparous group and  $r=0.024$  for the multiparous group).

The inference from this data analysis comparing primiparous to multiparous subjects is that whilst both groups changed, the change in the primiparous group was earlier and quicker than the multiparous group. Once again the clinical significance of this change is yet to be determined.

The explanations and thus the clinical significance of these results are unexplained in the literature review. It may be that there was a difference between subjects in the timing of subsequent pregnancies. If a woman has children close together will this influence the laxity change exhibited in the second pregnancy? The weight gain and the weight of the foetus at term may be significant in the way her tissues changed too. Further studies comparing primiparous and multiparous subjects would need to investigate the descriptive statistics carefully in order to examine these potential sources of error that might affect the results. Also increasing the numbers of subjects recruited will reduce the magnitude of any error observed.

The study has attempted to answer the research questions posed above and their potential clinical significance. When treating pregnant patients with back pain, knowledge of when in the pregnancy laxity changes might occur will lead to a different choice of manual techniques. For example a long lever technique may overstress the tissues at the end of the pregnancy. Likewise it would be better to restrict the treatments to shorter



treatments in order to avoid overstraining the tissues. Advice about rest and activity at different stages of the pregnancy too would be more accurate if there was some knowledge of when laxity changes might occur.

# ***Chapter Five:***

# ***The Post-Natal Study***

## **Introduction**

After studying the effects of pregnancy on peripheral joint laxity, it was felt that logically a study into laxity in the post-partum period should be undertaken next. Do women retain their laxity observed at the end of a pregnancy into the post-partum period or not? If they do retain joint laxity, what part does breast as opposed to bottle feeding have to play, if any? How quickly after having a child do women appear to return to pre-pregnancy laxity values?

These are the research questions the post-natal study addressed.

### **Literary review: Breast feeding and lactation.**

It is the practice of midwifery departments and health visitors to encourage breast feeding in new mothers if at all possible.

The Infant Feeding 2000 survey was conducted by BMRB Social Research, on behalf of the Department of Health, the Scottish Executive, the National Assembly for Wales and the Department of Health, Social Services and Public Safety in Northern Ireland. The survey provides national statistics on the incidence, prevalence, and duration of breastfeeding and other feeding practices adopted by mothers in the early weeks up to about nine months after birth.

According to the survey 69% of mothers breast fed at birth. As expected there was a general reduction in the proportion of mothers breast feeding

as babies got older. The proportion of mothers still feeding by the time the baby was six months old and nine months old respectively was down to 21% and 13% respectively, (Hamlyn et al., 2000).

In the Federal Drug Administration magazine of October 1995, Professor Ruth Lawrence from The University of Rochester School of Medicine is quoted as saying, "The academy recommends that babies be breast-fed for six to 12 months. The only acceptable alternative to breast milk is infant formula. Solid foods can be introduced when the baby is 4 to 6 months old, but a baby should drink breast milk or formula, not cow's milk, for a full year". She goes on to say, "There aren't any rules about when to stop breast-feeding; as long as the baby is eating age-appropriate solid foods, a mother may nurse a couple of years if she wishes. A baby needs breast milk for the first year of life, and then as long as desired after that" (Williams, 1995).

The FDA advice is given to women in the USA; in the third world breast milk is often the only food available for babies in their first few months of life. The idea of breast feeding being the best thing for the baby is promoted as being the natural thing to do. Certainly for a healthy baby the physiological effects of breast feeding from a well fed and healthy lactating mother are very difficult to challenge providing the mother wants to breast feed and is able to do so.

There are many reasons why mothers give up breast feeding or do not want to start or are unable to start in the first place. They include personal statements relating to how they felt about their own bodies or the convenience of bottle feeding to reasons related to the baby such as not latching on or failing to thrive. Nowhere is there any evidence that women fail to breast feed, or stop breast feeding, because they have musculo-skeletal pain.

Little research has been done on the adverse effects for the mother of breast feeding. What evidence there is of mothers being advised not to feed relates to either serious illness that may be passed on through the child through the milk such as AIDS, or it may be because the mother is taking medication that may pass through into the milk and thus adversely affect the child (Department of Health advice leaflet, 2004).

Post-partum joint pain is not a major factor in women's lives but it can be very real when present. Certainly anecdotally this author has encountered many women with back pain later in life who have related the onset of this pain to either pregnancy or the post-partum period.

A through search through the NLM and Medline data-bases failed to produce many papers that have explored laxity in peripheral joints at this time. One paper mentioned post-natal laxity in passing but it did not give a mechanism for this laxity and did not distinguish between those women

who breast feed their babies and those who do not (Schauberger et al., 1966).

Pelvic girdle pain, particularly pubalgia post-partum, has been studied in depth with varying conclusions being drawn from the work. In a study in Denmark in 2000, 1600 women entered the study and 2% reported post-partum pelvic pain after 12 months (Larsen et al., 2000). Similarly in a study in South Africa, 4.9% of a group of reported hypermobile women reported pain continuing into the post-partum period (van Dongen et al., 1999). There was little evidence shown in the studies attributing the post-partum pain to continuing laxity.

## **The physiology of lactation**

Lactation is controlled principally by the hormones prolactin and oxytocin produced by the anterior and posterior parts of the pituitary gland respectively. Oxytocin is present during the third stage of labour when it brings about the separation of the placenta and the contraction of the uterine muscles to prevent post-partum haemorrhage. It then continues to be secreted as a consequence of the infant's suckling and it aids the involution of the uterus and the expression of the milk (the so-called let down reflex). As the placenta is expelled, the circulating level of human chorionic gonadotrophin (HCG), human placental lactogen (HPL),

oestrogen, and progesterone all fall rapidly within the next forty eight hours (West et al., 1979; McNeilly 2001).

Prolactin acts on the hormones which control the fertility cycle. At the level of the pituitary gland it interferes with the action of gonadotrophin releasing hormone (GNRH) and lutenising hormone (LH). Thus there is no stimulation of the ovarian follicles and no ovulation during breast feeding and in this way the production of ovarian oestrogen in the form of oestradiol is inhibited (Rolland et al., 1975; McNeilly, 2001).

During lactation, most women experience a time of amenorrhoea. This is because of the effects of the circulating prolactin on the ovary and on the hypothalamus. Whilst she is breast-feeding, ovarian secretion of oestrogen and progesterone is below normal, and is equivalent to that seen in post-menopausal women in spite of normal levels of FSH (Rolland et al., 1975; Howie, 1993; Saarikoski, 1993; Jacob et al., 2004).

Complete weaning results in an immediate drop in the blood levels of prolactin and an increase in blood levels of LH and oestradiol, indicating a prompt resumption of ovarian activity. Ovulation usually occurs within 14 to 30 days. These results suggest that a maintained suckling stimulus, and the associated hyperprolactinaemia, suppress LH but not FSH post-partum and lead to both a failure of ovarian follicular development and lactational amenorrhoea (Jacob et al., 2004).

From the above then it is highly unlikely that the maintenance of ligamentous laxity during breast-feeding if it exists is due to the effects of oestrogen or progesterone because they are at sub-optimal levels.

At one stage relaxin was thought to have disappeared within forty eight hours post-partum with the loss of the corpus luteum of pregnancy and the placenta. However, recent studies seem to question this. In a study to determine if relaxin could be measured in milk, and if so correlate to concentrations in milk and serum (Eddie et al., 1989), paired samples of milk and serum were collected from sixteen women three days after pre-term delivery and from twelve women three days after term delivery. Eight term and six pre-term women were sampled again six weeks post-partum. At six weeks post-partum, relaxin was not measured in any sera but was measured in milk from six of the eight patients in the term group and five of six patients in the pre-term group. They concluded that the presence of relaxin in milk at six weeks post-partum when there is neither a corpus luteum of pregnancy nor any placental source, suggests a non-luteal site of synthesis. Current thought is that mammary tissue is the probable source of the relaxin from the breast tissue (Tregear, 2004). This finding has yet to be confirmed by other co-workers.

In 1992 one study looked at the effect of relaxin on oxytocin levels (Way and Leng, 1992) The researchers found that the injection of porcine



relaxin caused a sustained increase in circulating plasma oxytocin and vasopressin.

Another group a year later found a relationship between vasopressin and relaxin (Weisinger et al. 1993). In order to make milk women are in a state of negative water balance whereby they retain water in the tissues to contribute towards the milk production. A study into thirst and breast feeding (James et al., 1995) suggested a mechanism for the changes in osmolarity. They suggested that suckling sends nerve impulses to the paraventricular and supraoptic nuclei in the hypothalamus which may have afferents within the central nervous system which stimulates a thirst response simultaneous with oxytocin release. In theory the change in osmolarity means that breast-feeding mothers are "water logged" compared to the non breast-feeding mothers. This hypothesis is supported by a research group in 1994 (Wilson et al., 1994) who examined the effects of exogenous relaxin on oxytocin and vasopressin release. They found that when rats were injected with relaxin there was an increase in the levels of both oxytocin and vasopressin, but that the effects of oxytocin at the level of the mammary gland might be obscured by the action of vasopressin affecting blood flow to the gland. In other words the effect is central more than local., supporting the water retention hypothesis. If the water is to be retained the ideal place would be the loose interstitial tissues present at the end of pregnancy again due to the

relaxin effect, and the maintenance of breast-feeding therefore has the potential link with an increased joint mobility.

### **The aim of the post-natal study**

The aim of the post natal study was to investigate the potential change in laxity in breast and bottle feeding mothers.

### **The Hypotheses**

The main experimental hypothesis was that there is a difference in measured joint laxity in those mothers who breast fed their baby as opposed to those who bottle fed their baby.

The null hypothesis is that there is no difference between the two groups.

The second experimental hypothesis was that there would be a significant statistical difference in measured joint laxity when comparing the last pregnancy readings to the post natal reading in each group.

The second null hypothesis was that there is no significant difference when comparing the last pregnancy readings to the post-natal reading in each group.

## **Method and research protocol**

Continuous quantitative data were collected from 28 subjects using the hyperextensometer as described before. They were broken down into two groups of subjects, 17 of whom were breast-feeding their babies and 11 were bottle-feeding their babies. If a mother had started to breast-feed and then stopped in favour of bottle-feeding this was thought to be a confusing variable in the data and so she was rejected from the study.

They were measured from between 2 and 9 weeks post-partum with an average of 6 weeks post-partum. The subjects were studied when they came back to the author's osteopathic practice for a post-natal check up, or if they came back requiring treatment. All of the subjects had taken part in the pregnancy phase of the study.

## **Results and statistical analysis**

The data were analysed for normal distribution using a Shapiro-Wilk test.

The result showed the sample was unlikely to be from a normal distribution ( $p = 0.034$   $n = 28$ ), therefore non-parametric tests were used for the statistical analysis.

In order to investigate the first experimental hypothesis to compare the measured laxity between the breast and bottle-feeding groups post-natal.,

a Mann Whitney U test was applied to the data showing the results to be significant for a two tailed test ( $U = 137.5$ ;  $n_1 = 16$   $n_2 = 11$ ;  $p = 0.0117$ ).

Table 5: Descriptive statistics for the breast feeding group and the bottle feeding group

Breast Feeders		Bottle Feeders	
Weeks		Weeks	
Mean	6.375	Mean	5.363
Sr Dev	1.821	Sr Dev	1.567
SEM	0.455	SEM	0.472
Degrees		Degrees	
Mean	45.938	Mean	33.636
StDev	11.289	StDev	12.060
SEM	2.822	SEM	3.636

The mean laxity was significantly different between those subjects who breast fed their children and those who bottle fed their children. The null hypothesis was thus rejected.

The data were subjected to a Kruskal Wallis test and the result showed that there was no significant difference in variance between the measurements obtained from the breast feeding and the bottle feeding groups ( $p = 0.084$ ). As this result was not significant, a post hoc test was not considered necessary.

A correlation calculation for both groups showed little evidence of correlation between laxity and the number of weeks of breast or bottle

feeding respectively ( $p= 0.389$ ,  $r=-0.2887$ ,  $r^2= 0.0833$  for the bottle feeders and  $p= 0.864$ ,  $r= 0.0466$ ,  $r^2= 0.0021$  for the breast feeders).

In order to investigate the second experimental hypothesis looking at the potential difference in laxity change between the last pregnancy reading and the post natal reading, a Mann Whitney U test was applied to the data showing the results to be significant for a two-tailed test only in the bottle fed group, ( $U=78.5$ ,  $n=16$ ,  $p=0.058$  for the breast fed group;  $U=107.5$ ,  $n=11$ ,  $p=0.001$  for the bottle fed group). The experimental hypothesis is thus rejected.

A Kruskal-Wallis test showed that there was a significant difference in the variances when comparing the last laxity reading of pregnancy and the post-natal laxity reading in both breast and bottle feeding groups. The breast feeding result did not show a significant difference in variance ( $p= 0.621$ ) whilst the bottle feeding group did ( $p= 0.0002$ ).

A correlation calculation found that there was no significant correlation between laxity readings at the end of the pregnancy and the laxity readings post natal in either group ( $r=0.4348$ ,  $r^2=0.1891$ ,  $p=0.0923$  for the breast feeding group;  $r=0.595$ ,  $r^2=0.350$ ,  $p=0.0835$ ).

## **Discussion and conclusions**

The results draw mixed conclusions from the data. This was probably because of the very small number of subjects available to test. If the study were to be repeated then larger numbers would hopefully give more meaningful data.

The literature review showed the importance given to breast feeding in the current information available to mothers. It states that breast feeding can continue until nine months post-partum and beyond. If there is an effect on ligamentous laxity, such that the longer she feeds the greater the effect on laxity then obviously there is a need for more information to support the statement. This would be especially so if she was suffering from post-partum back pain and it was felt that ligamentous laxity was contributing to the problem. There is insufficient evidence available to reliably inform patients that the continuation of breast feeding is having a deleterious effect on her tissues. If her body is producing relaxin from a non ovarian source (Eddie et al., 1989), then the effects if any of this relaxin on post-natal ligamentous laxity have yet to be determined.

# ***Chapter Six:***

# ***The Menopause Study***

## ***Introduction***

The final study in this series examines two groups of women after the menopause. One group was using a prescribed HRT preparation, and the other group, the control group, was not. The aim of the study was to investigate if the use of HRT had any effect on the joint laxity in the groups under study.

The menopause is a time in women's lives when changes to their connective tissues occur due to oestrogen withdrawal. These changes can be relatively minor changes to the skin and muscular tissues, or they can involve muscular weakness of the pelvic floor leading to incontinence and vaginal prolapse. As women get older they are liable to the effects of osteoporosis in bone, and degenerative changes in the connective tissues around their joints leading to osteoarthritis (Spector et al., 1997). The following paragraphs review the role of the sex hormones on the connective tissues at this time.

## **Literary Review: The Physiology of Menopause**

Clinically, menopause is defined as the cessation of menstrual cycles and results from either follicular depletion, a "natural" menopause or surgical removal of the ovaries defined as an "induced," or "surgical,"



menopause (van Noord et al., 1997). However, apart from surgical interventions, menstrual cycles seldom cease abruptly; there is an interval termed the "peri-menopause" or "menopausal transition," during which there are considerable hormonal fluctuations (Lobo, 2000). The peri-menopause usually begins a few years before the last menstrual cycle; the cycles become irregular, and there are often symptoms suggesting a decline in oestrogen concentration. Oestrogen levels can even swing higher than normal early in the peri-menopause, but an abrupt decline in oestrogen occurs 6 months before menopause (Lobo, 2000).

The peri-menopause also extends for a few years after the last menstrual cycle; during this time, transient and episodic bursts of ovarian activity may occur, which may result in some vaginal bleeding (Lobo, 2000).

Natural menopause occurs in western Europe at a median age of 51.4 years, with a distribution ranging from 40-58 years (van Noord et al., 1997). The age at onset of natural menopause and the risk for surgical menopause both seem to be determined by familial factors as well as by genetic polymorphisms of the oestrogen receptor (ER) (Weel et al., 1999). Multiparity and increased body mass index (BMI) are associated with later onset (Kato et al., 1998; Hardy, 1999), whereas smoking (Cramer et al., 1995a; Windham et al., 1999), null parity and medically treated depression, (Harlow et al., 1995), toxic chemical exposure, (Silbergeld 1998) and treatment of childhood cancer with abdominal-pelvic radiation

and alkylating agents (Chiarelli et al., 1999) have been associated with a younger age at onset.

All of these factors are important in themselves and in relation to the woman's total physiology. One of the linking factors between the decline of the sex hormones and the global effects of the menopause are the effects of the declining hormone levels on the collagenous connective tissues that form a fibrous connective tissue "skeleton" supporting all of the body's structures. The connective tissues, and in particular collagen, support and envelop the organs in the abdomen and pelvis, as well as smooth and skeletal muscle in both form and strength throughout the whole body. The following paragraphs investigate the changes occurring during the menopause and the effects in particular of the decline in the levels of the sex hormones on the function of ligaments and muscular tissues.

## **Hormonal Changes and the Menopause**

The typical hormonal changes in the early follicular phase of postmenopausal women compared with those of ovulatory women are shown in table 6. Compared with the typical hormonal changes in the early follicular phase of ovulatory women, in post-menopausal women, the most significant findings are the marked reductions in oestradiol (E2) and oestrone (E1) levels. The serum E2 level is lower than the serum E1

level. Serum E1 is produced primarily by peripheral aromatization of androgens, which are not as dramatically affected by menopause. Apart from elevations in FSH and lutenising hormone (LH) occurring in response to the oestrogen decline, pituitary hormones are not affected. Specifically, growth hormone, thyroid-stimulating hormone, and adrenocorticotrophic hormone levels are normal.

Table 6: Serum sex steroid concentration before and after natural and surgical menopause.

From Hormone Replacement Therapy by Whitehead and Godfree Churchill Livingstone 1993

	Oestrone E1	Oestradiol E2	Progesterone	Testosterone	Androstenedione
	pmol/l	pmol/l	Nmol/l	nmol/l	nmol/l
<b>Premenopausal</b>					
Early follicular	90-180	90-350	0.3-1.6	0.7-1.4	5.6-6.1
Late follicular	550-740	740-1390	0	1.0-2.8	6.4-7.0
Mid luteal	260-370	520-870	>20-30	1.0-2.1	0
<b>Postmenopausal</b>					
Natural	75-150	35-55	0.3-0.8	0.7-1.0	2.1-3.1
Surgical	75-150	35-55	0.3-0.8	0.3-0.6	1.7-2.8

Serum prolactin levels may be very slightly decreased because prolactin is influenced by oestrogen status. Both the post-menopausal ovary and the adrenal gland continue to produce androgens.

Changes in mobility after the menopause are related to degeneration of the joint surfaces that control the ability of the joints to move, the supporting and moving skeletal muscles, both in bulk and in muscle strength, and also the supporting connective tissues in the joint ligaments and capsules.

## **The Effects of Declining Oestrogen**

Oestrogen receptors are abundant throughout the body as has already been seen in a previous the menstrual cycle chapter. The menopausal decline of oestrogen leads to a decline in oestrogen stimulated effects on a wide range of tissues other than in the genital tract. Oestrogen receptors are abundant in the brain (Shughrue et al., 1997) and oestrogen is known to have a role in many brain processes, with the withdrawal of oestrogen resulting in physiological and symptomatic changes.

Oestrogen is important for cerebral blood flow, cerebral glucose administration, synaptic activity, neuronal growth, the survival of cholinergic neurons, as well as such complex functions as cognition (Saravia et al., 2004; Lu et al., 2004; Pfaff, 2004).

## **The decline of oestrogen and its effects specifically on collagen**

Oestrogen levels have a positive effect on the stimulation of fibroblasts to produce collagen, which is important for bone and skin (Bienkiewicz et al., 1966; Rajabi et al., 1991). Both oestrogen and androgen receptors have been identified in skin fibroblasts (Harvey et al., 1976). The loss of collagen is more rapid in the first few years after menopause, and 30% of skin collagen is lost within the first 5 years after menopause (Falconer et al., 1996; Reay Jones et al., 2003).

The rate of collagen decrease is approximately 2% per year for the first 10 years after menopause. This statistic, which is similar to that of bone loss after menopause, strongly suggests a link between skin thickness, bone loss, and the risk of osteoporosis. In addition, reductions in collagen support and atrophy of the vaginal and urethral mucosa have been associated with a variety of symptoms, including uterine prolapse and urinary incontinence (Slemenda et al., 1987). Therefore the loss of oestrogen receptors after the menopause has a profound effect on collagen and is an important reason why some patients are prescribed HRT.

### **The decline of oestrogen and its effects specifically on skeletal muscle**

In the literature review in the chapter on the menstrual cycle study, ample evidence was presented to suggest a strong link between phases of the menstrual cycle and muscle performance.

There have been many studies that have investigated the effects of HRT and muscle strength after the menopause. A paper in 2005 suggested that muscle strength begins to decline during the peri-menopausal years, and this phenomenon is partly oestrogen dependant (Sirola and Rikkonen, 2005). They go on to state that hormone replacement therapy may prevent a decline in muscle performance. Patients must take the hormone over an extended period if the effects are to work. In a paper published in 2002, 40 post-menopausal women were randomly divided into two groups. The study looked at hand strength, isokinetic knee flexion and extension, and general physical activity, and the subjects were measured before treatment and after three and six months. Their data suggest that the patients had to use HRT for longer than six months for the muscle strength to be affected (Ribom et al., 2002). However, the underlying mechanism of HRT action on muscle is still unclear as there is considerable variation in the effects of HRT between different studies (Siplia, 2003).

As women get older they are going to become more susceptible to degenerative changes in their joints and in their supporting structures. There have been studies that have linked degenerative change and HRT (Spector et al., 1997) and they have concluded that HRT can reduce the effects of osteoarthritis in peripheral joints. The changes in laxity in particular have yet to be studied, and the effects of this laxity continuing into the later years of a woman's life again have yet to be researched.

## **The Hypotheses**

The experimental hypothesis states that there is a difference in joint laxity between those subjects who take an HRT preparation after the menopause and a control group. The null hypothesis states that is no difference in joint laxity after the menopause between women who take HRT and a control group.

## **Ethical considerations**

All subjects were given an appropriate information sheet about the relevant study for them, and they were required to sign a consent form a copy of which was given to them to keep. Ethical approval for the studies had been obtained from the Ethics Committee of The British School of Osteopathy.

## **Method and research protocol**

Continuous quantitative data were collected using the Hyperextensometer, as described in the previous chapters, from 50 female subjects. The subjects were obtained from the author's private

practice and from a menopause clinic at a neighbouring GP practice. One group of 25 subjects used Hormone Replacement Therapy (HRT) in a combined oestrogen and progesterone preparation and the other group of 25 subjects did not use any HRT.

Hormone replacement therapy can be used in many forms and it was felt that this may well be an important potential confounding variable to be considered. Therefore only those women using a combined oral preparation were accepted for measurement on the study.

The average ages of the groups were 54.44 years in the HRT group and 54.88 in the non-HRT group.

The patients at the GP practice were diagnosed as being post-menopausal by a blood test for those experiencing menopausal type symptoms. These included amenorrhoea, vaso-vagal symptoms and hot flushes, and changes in connective tissues including vaginal mucosal drying. Those who were diagnosed as being menopausal were then offered HRT in various forms. On average the patients in the HRT group had been taking the preparations for 1 year and three months.

The women were measured at weekly intervals on three consecutive weeks.



Results and statistical analysis

The data were analysed for normal distribution using a Shapiro-Wilk test. The result showed the sample was unlikely to be from a normal distribution ( $p= 0.0095$   $n= 150$ ) therefore non-parametric tests were used for the statistical analysis.

Table 7: Descriptive statistics for the HRT and non HRT groups

	Reading One		Reading Two		Reading Three	
	HRT	Non HRT	HRT	Non HRT	HRT	Non HRT
Mean	52.08	39.72	53.84	38.88	54.84	40.06
St Dev	19.31	13.48	18.7	13.24	18.69	13.77
SEM	3.86	2.7	3.74	2.65	3.74	2.75

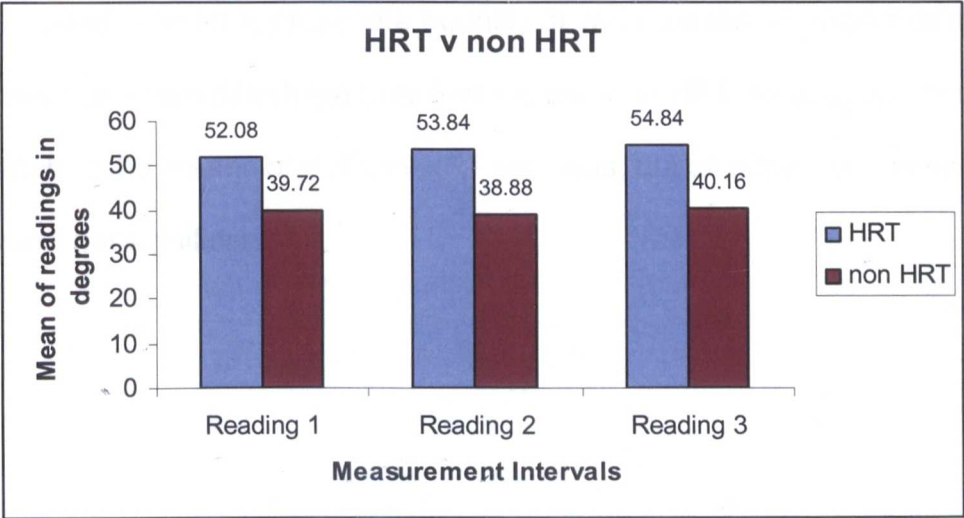


Figure 33: The mean readings for the HRT and non HRT groups

Figure 32 shows the mean readings taken from the HRT and non-HRT groups over three weeks. It clearly shows that there is a difference between the two groups. In order to investigate the significance of this difference and to examine the experimental hypothesis, a Mann Whitney U test was applied to the data showing the results to be significant for a two tailed test ( $U = 3971.5$ ;  $n_1 = 75$   $n_2 = 75$ ;  $p = 0.0001$ ).

The mean laxity was significantly different between the HRT and the non HRT groups, and the null hypothesis was thus rejected.

In order to ascertain where the differences lay, the data was subjected to a Kruskal-Wallis test to analyse the variances between the two groups. The result showed that there was a highly significant difference in variance between the group using HRT and the control group ( $p = 0.0016$ ).

A squared ranks approximate equality of variance test showed that there was significant difference between the mean of HRT reading one and each of the means of the non-HRT readings, but all other comparisons were not significant.

## Discussion and Conclusions

The results clearly show that the experimental group did show a significant increase in joint laxity as compared to the control group. It appeared that the change was consistent across the three readings. The menopause represents a milestone in female health and, after passing through it, women experience increased musculo-skeletal and cardiovascular morbidity. One study from Oslo maintained that older age, higher morbidity, and lower functional capacity are associated with fall injuries. The inability to get up from the floor has been associated with older age, higher morbidity and lower functional capacity. If a patient was unable to get up from the floor it is a marker of failing health and a useful predictor of serious fall injuries (Bergland et al., 2005). Muscle and joint performance is an important determinant of functional capacity and quality of life among the elderly and is involved in the maintenance of balance.

A study from the Netherlands investigated the relationship between duration and intensity of physical activity and disability ten years later. It concluded that a physically active lifestyle was inversely proportional to disability (van den Brink et al., 2005). If elderly patients suffer from degenerative diseases of the weight-bearing joints then they tend to sit longer and not challenge their joints rather than walk and suffer pain. This can have a negative effect on their cardio-pulmonary systems. A paper

from Denmark looked at outdoor activity performance in patients suffering from chronic obstructive pulmonary disease. Patients reporting on their outdoor activity (n= 148), were compared to immobile patients. They reported that the mobile group of patients had higher performance status, higher body mass index, and lower duration of oxygen administration, than the immobile group (Ringbaek, 2005).

The menopausal study showed a retained laxity, or reduced stiffness, in the experimental group as compared to the control group. This laxity allows patients a greater mobility and thus a better quality of life alongside the muscle strength changes mentioned in the literature review. Paradoxically it may lead on to degenerative changes in the joints because they continue to be used for strenuous activities much later. The early stage of degeneration of the synovial joints involves a phase of hypermobility or joint looseness as the depth of the articular cartilage is reduced (van der Esch et al., 2005). It may be that the measured joint laxity reflected this early change.

In clinical practice, the assessment of joint stiffness as well as joint pain is used as a diagnostic feature when treating patients with degeneration of their synovial joints. It is this author's opinion that knowledge of their medication as it relates to the use of HRT may influence the assessment of normal mobility in these patients.

The inclusion of HRT as part of a raft of measures used in the treatment of degenerative joint disease in elderly women must be weighed against the potential risk factors. If it has been seen to have a positive effect on joint mobility as well as muscle strength then it may well be a factor worthy of consideration in the mind of the prescribing clinician.

***Chapter Seven:***  
***Main findings and***  
***opportunities for***  
***future work***

## **Summary of findings**

The results from the questionnaire revealed that there was a strong link between the menstrual cycle and the timing, the location, and the chronicity of pain. It was very interesting to note that there were two peaks relating to the onset of a patient's pain, one corresponding to ovulation and one at the end of the cycle corresponding to the premenstrual phase of the cycle. Low-back pain and cervical spine pain were both present at these times in the cycle. What was surprising was that upper extremity pain appeared in the middle of the cycle and lower extremity pain at the end of the cycle. The explanation for these findings was not clear. However, what was clear from the study was the similarity of evidence of injuries that occur in athletes with the responses from the questionnaire.

The menstrual cycle study gave clear evidence that laxity changes during the menstrual cycle, and that the level of oestrogen was significant to this change. There was not clear evidence linking other hormones to changes in joint laxity.

The pregnancy study showed that as expected from the literary review, changes in laxity occur during pregnancy, in both primiparous and multiparous women. What was different about this study was the rate of change of that laxity. It was not uniform throughout the pregnancy and

there did not appear to be a clear correlation between her laxity at the start of her measurements and the rate of change. One might have expected a subject who was clearly hypermobile at the start of her measurements as compared to the rest of the group, to be even more so at the end of the pregnancy, but this was apparently not so. Likewise very stiff subjects when compared to other subjects at the start of their measurements did not have a clearly different rate of change. The study did not have the opportunity to measure hormone levels in these women and therefore to correlate the changes in levels of oestrogen and relaxin with joint changes as it did in the menstrual cycle study. This was unfortunate as it would have provided clear data on which hormones appear and when in relation to the laxity change of pregnancy. Johnson's group in 1991 and 1994 has shown that relaxin in both IVF and natural conceptions appears to be present at the end of the pregnancy, but information as to its first appearances is still unclear (Johnson et al., 1992, 1994).

Unfortunately the post-natal study did not reveal a definite conclusion. The small numbers of subjects involved who returned to be checked meant that there was not sufficient data to give a clear picture of the laxity changes. If the study were to be repeated, then firstly the importance of coming for the post-natal check at six weeks post-partum could be emphasised to the participants which might encourage them to make the effort to return to be measured. Alternatively if the subjects in the



pregnancy and in the post-natal study came from an antenatal clinic either in a hospital or in a GP practice, then they could be approached to be measured when they attended for their six weeks post-natal obstetric check.

The menopausal study again revealed a clear link between the increased laxities in the experimental group when compared to the control group. The implications of this that relate to the development of degenerative changes in the weight-bearing joints are thought to be very interesting, and they could be another factor to be borne in mind by a clinician who was making the decision to prescribe HRT for a patient or not.

### **Further research opportunities**

The questionnaire is worthy of refining and repeating. More attention to the wording of the questions relating to the site of pain and giving more groups for the patient to choose from would lead to greater specificity in defining and describing the painful area. Also if the questionnaire could be designed such that the patient filled out the form instead of the practitioner it may well remove an element of bias from the study. The use of tick boxes could be helpful here. The use of a visual analogue scale would give an indication of the severity of the pain and thus add weight to the questions relating to the nature of the pain.

The menstrual cycle study could be repeated with greater numbers without too much difficulty. If it was repeated in a institution such as a university hall of residence, a military facility or a prison where the population was fixed and resident, the subjects would be available for measuring on a daily basis over a number of consecutive menstrual cycles. The levels of oestrogen could be obtained from urine samples, however, the levels of progesterone and relaxin would be much more difficult to obtain as they do not appear in the urine. Repeated daily serum samples are the only reliable way to obtain data about these hormones, but this is a very invasive procedure and would require careful ethical controls. Perhaps the collection of data in conjunction with an IVF unit where women's blood was being measured on a daily basis for other reasons would be a more practical solution. Following subjects over three or more cycles would give a much clearer picture of what is happening from cycle to cycle, giving the researchers a clearer understanding of just how representative the data obtained from the menstrual cycle are.

The pregnancy study could be repeated when the women are going for routine blood tests anyway during the pregnancy and this would give a clearer picture of the changes occurring during pregnancy.

In each of the studies more subjects measured would give more data to be analysed and thus a clearer view of the laxity changes at each stage in a woman's reproductive life.

The Hyperextensometer proved to be a reliable and cheap way of examining the laxity at the first metacarpo- phalangeal joint with little distress or inconvenience to the subjects being measured. For this reason I would recommend its use again in this type of research.

# SUMMARY

## THE PREGNANCY STUDY

1. Confirmed that all subjects experienced changes in laxity during pregnancy.
2. The rate of change was not uniform and there was no clear correlation between hyper and hypo laxity at the start of the pregnancy and laxity changes that occurred later on.
3. Laxity change was most apparent in the middle months of the pregnancy not at the end

## THE PILOT STUDY

Demonstrated that women Experienced musculo-skeletal pain at specific times within the menstrual cycle ; notably on day 14 and on day 27 of a 28 day cycle

## THE MENSTRUAL CYCLE STUDY

1. Demonstrated that laxity changed in every female every month, but not in the male subjects used as a control group.
2. Oestrogen was the most important hormone in the middle of the month and relaxin at the end of the month.
3. These changes were temporary and reversible by the start of the next menstruation.

## THE POST NATAL STUDY

Failed to show a correlation between laxity ,breast feeding mothers ,and bottle

## THE MENOPAUSAL STUDY

Demonstrated that changes in laxity were greater in a group of post menopausal women using HRT than a control group who did not. Oestrogen again was the predominate hormone responsible for this change

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***The Appendices***

## **Appendix One**

### **The Histology and Physiology of Collagen**

Of all of the non-mineral constituents of the mammalian body there is more collagen than anything else other than water and possibly fat.

Nevertheless our understanding of the physiology of collagen is rudimentary (Rennie MJ 1999).

All cells and tissues are supported by a network of collagen fibres, the arrangement of which appears to be specifically site adaptive. All collagen molecules are made within fibroblasts (or modifications of them such as osteocytes). The large collagen molecule is secreted in a soluble form, with hydrophilic ends which are enzymatically cleaved to leave the insoluble core collagen, called tropocollagen, in the extra cellular space.

Collagen is has a high tensile strength due to the cross linking which makes it resistant to many proteases other than collagenase.

Collagens are a family of closely related proteins and are the most abundant and important extra cellular fibrillar proteins.

The main role of collagen is to provide tensile strength to tissues by forming collagen fibres; in addition one type of collagen forms the structural scaffold of basement membranes.

Fibrillar collagen is formed from three polypeptide (alpha ) chains consisting of repeating sequences of 3 amino acids, 2 of which are

proline or lysine or glycine and the third another. These are initially secreted with both amino and carboxyl terminal extensions to prevent collagen forming inside cells. Initial assembly of these chains is into a triple helix called procollagen.

Cleavage of the amino and carboxyl extensions to leave the functional mid section, tropocollagen, allows the molecules to align themselves into linear arrays to form long filaments. The individual collagen molecules are 300 nm long, and give rise to a periodicity of 67 nm.

The initial filaments or collagen micro-fibrils become arranged into fibrils are arranged with a 67 nm overlap between adjacent molecules. This and the fibres into larger bundles by tight cross linking between adjacent molecules via lysine residues; this contributes to the mechanical strength of collagen in tissues.

There are at least 20 different types of alpha chain which combine to produce different forms of collagen. Type I II and III collagen are arranged as rope like fibrils and are the main forms of fibrillar collagen.

Collagen fibres have a high tensile strength, thus their orientation and cross linking varies according to the local environment.

Reticular fibres (also called reticulin) are thin fibrils (20nm in diameter) of type III Collagen They form a loose mesh in many support tissues and are particularly evident in a zone beneath basement membranes where they are thought to have a support function as part of the fibro-reticular lamina.

Reticular fibres can be considered fine scaffolding supporting specialised extra cellular matrix components. In lymph node, spleen, and bone marrow, they fibres form the main extra cellular matrix fibres supporting the haemopoietic and lymphoid tissues. In parenchymal organs, such as the liver and kidneys, reticular fibres form a network supporting specialised epithelial cells.

Type	I	II	III	IV	V	VI
Morphology	Large banded collagen fibre	small banded collagen fibre	small banded collagen fibre	sheet like layers	thin fibrils	thin fibrils
Distribution	skin dermis, tendon, bone,ligaments, fascia, fibrous cartilage, cornea, loose connective tissue	hyaline and elastic cartilage, vertebral discs, vitrious of the eye	blood vessels, parenchymal organs,bone marrow, lymphoid tissue, smooth muscle, nerves,lung, foetal skin	basement membranes, external laminae lens capsule	basement membranes of placenta, smooth and skeletal muscle	ubiquitous
Type	VII	VIII	IX	X	XI	
	short striated fibrils	uncertain	uncertain	uncertain	uncertain	
	anchoring fibrils in basement membrane of skin and amnion	endothelium	cartilage	mineralising cartilage	cartilage	

Figure 34: Molecular Forms of Collagen from Histology by Stevens and Lowe (Stevens A and Lowe J 1992)

Type IV collagen assembles into a mesh network rather than fibrils and is restricted to basement membrane formation.

Type VII collagen forms the anchoring fibres of some basement membranes.

Although the main cells producing collagen are fibroblasts, it can also be produced by other mesenchyme derived cells (of the support cell family,) as well as by a variety of epithelial and endothelial cells which produce the type IV collagen of basement membranes.

Tendons consist of collagen (mostly type I collagen) and elastin embedded in a proteoglycan-water matrix with collagen accounting for 65-80% and elastin approximately 1-2% of the dry mass of the tendon. These elements are produced by tenoblasts and tenocytes, which are the elongated fibroblasts and fibrocytes that lie between the collagen fibres, and are organized in a complex hierarchical scheme to form the tendon proper. Soluble tropocollagen molecules form cross-links to create insoluble collagen molecules which then aggregate progressively into microfibrils and then into clearly visible units when viewed with an electron microscope, these are the collagen fibrils.

A bunch of collagen fibrils forms a collagen fibre, which is the basic unit of a tendon.

A fine sheath of connective tissue called endotenon invests each collagen fibre and binds fibres together. A bunch of collagen fibres forms a primary fibre bundle, and a group of primary fibre bundles forms a secondary fibre bundle. A group of secondary fibre bundles, in turn, forms a tertiary bundle, and the tertiary bundles make up the tendon. The entire tendon is surrounded by a fine connective tissue sheath called epitenon.

The three-dimensional ultra structure of tendon fibres and fibre bundles is complex. Within one collagen fibre, the fibrils are oriented not only



longitudinally but also transversely and horizontally. The longitudinal fibres do not run only parallel but also cross each other, forming spirals. Some of the individual fibrils and fibril groups form spiral-type plaits. The basic function of the tendon is to transmit the force created by the muscle to the bone, and, in this way, make joint movement possible. The complex macro- and microstructure of tendons and tendon fibres make this possible. During various phases of movements, the tendons are exposed not only to longitudinal but also to transversal and rotational forces. In addition, they must be prepared to withstand direct contusions and pressures.

This description of the three-dimensional internal structure of the fibres forms a buffer medium against forces of various directions, thus preventing damage and disconnection of the fibres (Kannus, 2000).

## Appendix Two

### Muscle fibre types

Different muscles are characterised by different physiological and metabolic properties, which are determined by differences in the structure of their constituent muscle fibres. In both animals and man it has been possible to define several sub types of muscle fibre by macroscopic, physiological., biochemical and histological criteria. Histochemical staining for specific enzymes delineates several types of fibre. Two main types of fibre are identified, the type 1 and the type 2 fibres. Type 2 fibres are further subdivided into types 2A 2B and 2C. Not all muscles have the same proportion of type 1 and type 2 fibres. In general., muscles with a role in maintaining posture such as the calf muscles have a higher proportion of type 1 fibres and are designated “fast twitch” fibres. While muscles used for short bursts of power are designated “slow twitch” fibres.

Fibre Type	Colour	Metabolism	Contractile behaviour
1	intermediate	oxidative	Slow twitch
2A	red	Oxidative and glycolytic	Fast twitch fatigue resistant
2B	white	glycolytic	Fast twitch fatigue sensitive

Figure 35: The Physiological features of different skeletal muscle fibre types

Comparison of fast twitch (the gastrocnemius/plantaris complex) and slow twitch (soleus) muscles revealed that the latter contained higher concentrations (expressed per gram of tissue wet weight) of glucocorticoid and oestrogen receptors but not androgen receptors.

Expressed per milligram of soluble protein, the slow twitch fibres contained higher concentrations of all three receptors. When related to the concentration of DNA, only the concentration of oestrogen receptors was higher in the slow twitch muscles.

Different response of the two fibre types to the direct action of the steroid hormones can therefore be expected. The fast twitch muscle contained a higher concentration of soluble protein, whereas the slow twitch muscle contained a higher concentration of DNA, resulting in lower protein /DNA ratio i.e. smaller cell units in the slow twitch muscles, soleus v gastrocnemius.

## **Information leaflet for the questionnaire**

My name is Stephen Sandler and I am an osteopath registered with The General Council and Register of Osteopaths.

I am currently engaged in researching into women's musculo skeletal injuries in relation to the timing of the menstrual cycle.

This will involve volunteers being asked a short series of questions by your osteopath prior to your treatment. The questions will ask you about the length of your menstrual cycle. Only those women who have a regular and predictable menstrual cycle are being asked to take part in this research. Also only those women who are not using any hormone preparations such as Hormone Replacement Therapy (HRT), an oral contraceptive, or hormone implants are being asked to take part in the study because the menstrual cycles of those patients is not under the control of a natural hormone profile.

The whole thing will take less than five minutes and the results are kept anonymous so that individual responses cannot be identified. When your osteopath has collected the data they will be sent back to me.

If for any reason you decline to take part in the study please tell the osteopath who will then go on to choose someone else.

Ethical approval for the study has been given by the Ethical Research Committee at The British School of Osteopathy.

Thank you

## **Information leaflet for menstrual cycle study:**

My name is Stephen Sandler and I am an osteopath in private practice registered with the General Osteopathic Council.

I am currently engaged in a research project that is investigating laxity in ligaments in women of various ages.

The project will involve you being measured at specific times over the next few weeks. The measurement involves you having your finger measured for joint looseness. It is a painless procedure that takes just a few seconds.

At the same time as the joint measurement I will need to take a small sample of blood from a vein in your arm. This blood will be analysed at the Chelsea and Westminster Hospital for levels of the hormones Oestrogen, Progesterone and Relaxin which are present during your menstrual cycle. All samples will be destroyed after the results have been obtained. In this way it is hoped that I will be able to see if there is a change in the looseness of the finger joint which corresponds to changes in hormones present in a woman's menstrual cycle. You do not need to undress for the procedure to be performed, and it will take place at a time and place convenient to you. You are free to withdraw from the study at any time and all of the results from the study will be printed such that they are completely anonymous.

Ethical approval for the study has been given both by the Ethical Research Committee at the British School of Osteopathy and at the

Chelsea and Westminster Hospital who will analyse the blood at the end of the study.

If you feel that you are happy to take part in the study please sign and date the attached consent form.

Thank you

## **Information leaflet for the men involved as the control group for the menstrual cycle study:**

My name is Stephen Sandler and I am an osteopath in private practice registered with the General Osteopathic Council.

I am currently engaged in a research project that is investigating laxity in ligaments in women of various ages. Men are being measured too as a control group because the main hypothesis of the study is that it is the level of circulating female hormones that has a part to play in changing laxity in women's joints. If the hypothesis is correct the men in the control group will not change.

The project will involve you being measured at specific times over the next few weeks. The measurement involves you having your finger measured for joint looseness. It is a painless procedure that takes just a few seconds. You do not need to undress for the procedure to be performed, and it will take place at a time and place convenient to you.

You are free to withdraw from the study at any time and all of the results from the study will be printed such that they are completely anonymous.

Ethical approval for the study has been given by the Ethical Research Committee at The British School of Osteopathy.

If you feel that you are happy to take part in the study please sign and date the attached consent form.

Thank you

## **Information leaflet for the pregnancy, post natal and the menopause studies:**

My name is Stephen Sandler and I am an osteopath in private practice registered with the General Osteopathic Council.

I am currently engaged in a research project that is investigating laxity in ligaments in women of various ages.

The project will involve you being measured at specific times over the next few weeks. The measurement involves you having your finger measured for joint looseness. It is a painless procedure that takes just a few seconds. You do not need to undress for the procedure to be performed, and it will take place at a time and place convenient to you.

You are free to withdraw from the study at any time and all of the results from the study will be printed such that they are completely anonymous.

Ethical approval for the study has been given by the Ethical Research Committee at The British School of Osteopathy.

If you feel that you are happy to take part in the study please sign and date the attached consent form.

Thank you



**Consent Form:**

NAME.....

ADDRESS.....

.....

.....

.....

I hereby give my informed consent to take part in the study entitled

.....

Which I understand is a research study approved by the Ethics  
Committee of The British School of Osteopathy and of the Chelsea and  
Westminster Hospital.

I have read and had explained to me the attached information leaflet and  
I understand that I can withdraw from the study at any time.

Signed.....

Dated.....